

UNITED STATES DEPARTMENT OF THE INTERIOR
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THE PROGRESSIVE FISH-CULTURIST

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AN EXPLANATION OF THE PRINCIPLES AND METHODS OF TISSUE CULTURE

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TISSUE CULTURE is the name applied to a group of loosely related techniques wherein cells or tissues from the higher plants and animals are kept alive, and in some cases growing, after separation from their source. Tissue culture has already found application in fish disease research. The future will bring many reports of investigations in which this new tool will be applied. This communication is intended to familiarize the reader with tissue culture and how it is used.

Culture of tissues in glassware is a comparatively new biological technique. Credit for the innovation is usually given to Roux, who, in 1885, placed part of the brain of a chick embryo in warm saline and watched its further development for a brief period. As time went by, the methods were improved so that tissues could be kept alive for weeks. About 30 years ago Alexis Carrel introduced concepts, techniques, and apparatus which form the basis for much of today's work with tissue culture (Parker 1950). Since then, the development of tissue culture has been rapid, and the number of uses to which it has been put has mushroomed. The Salk polio vaccine is probably the best-known product of applied tissue culture. Its international distribution and use would probably not have been possible at this time had it not been for the methods of growing the virus in tissue culture. This, however, is but one use; cultures of living cells are indispensable for such diverse studies as embryology, cytology, histol-

ogy, radiation effects, nutrition, physiology, immunology, cancer investigations, and virology.

The popularity of tissue culture is well deserved. It is often the most convenient, and in some instances the only, method available for certain studies. Tissue culture can also provide highly uniform biological units at low cost. As an example, thousands of cultures may be prepared from one laboratory animal, and each culture can be used, for some work, in place of the animal. Many cultures are prepared for microscopic observation; and the progress of cellular growth, degeneration, alteration, or other change can be watched and photographed. This is usually impractical when laboratory animals are used. Cultures of some cells are propagated with methods similar to those used to grow bacteria; prepared cultures of certain cells may even be bought from a commercial source. This feature makes it possible to use the same cell type for thousands of trials year after year.

How, then, are the tissues or cells successfully cultured? The principles by which living tissues are grown in glassware can be simply stated: the techniques must furnish the essential needs of living protoplasm. Within the glassware, the tissue must find nutritive substances, respiratory gases, and favorable environmental factors such as temperature and pH. It must have protection from disease and be provided with a system or method of removing metabolic wastes.

ESSENTIALS FOR TISSUE CULTURES

Nutrition

The higher living animal eats a variety of foods, but actual utilization of this food requires modification to substances which can be dissolved in and carried by the blood stream. The cells that compose a fish, for example, are nourished by nutrients that diffuse from or are absorbed from the fluid part of the blood. In this way a fish metabolizes minerals, amino acids, vitamins, carbohydrates, fatty acids, growth factors, and the like.

The nourishment of cells in a test tube is maintained in just this way. Blood is collected, and the fluid phase is separated and used to "feed" tissue cultures. There are complications in some instances. It is often difficult to obtain adequate supplies of blood serum from small animals such as mice. Those who work with tissues from some insects would find their task insurmountable if it were not possible to use blood serum from other animals of larger size. Fortunately, most tissues do not have an absolute requirement for homologous serum (the liquid part of the blood), and sera from larger animals such as sheep, horses, and cows will serve the purpose adequately.

Many different sera are available from biological supply houses. Partly for reasons of economy, salt solutions that contain the correct proportions of inorganic chemicals found in blood serum are usually used to dilute and extend the serum. This, of course, suggests a synthetic serum; and progress has been made toward developing completely synthetic nutrients for use in tissue culture. It is still not possible, however, to maintain cultures for long periods without the addition of some natural serum.

Excretion

Living cells give off metabolic wastes which must be removed lest they accumulate and become toxic. The animal kidney filters out much of this material, but culture systems have no kidney and must

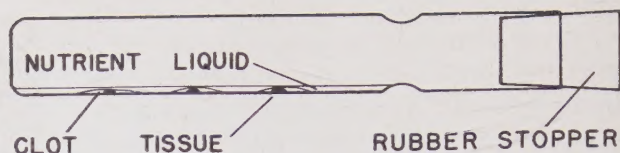


FIGURE 1.— Schematic cross-section of Carrel culture flask containing tissues embedded in plasma clot and submerged in a nutrient fluid. The neck of the flask is constricted at its base to form a dam that keeps the nutrient within the body of the flask.

incorporate some provisions for "excretion." The relatively large volume of nutrient that surrounds tissues in culture (figure 1) dilutes and buffers much of these metabolic wastes, but its capacity is limited. In practice, these wastes are periodically eliminated by removing the old medium and replacing it with fresh. The method is somewhat wasteful, and this is another reason why nutrient materials are diluted with salt solutions.

Respiration

Living tissues must be provided with an exchange of gases. Oxygen must usually be available; and as the atmosphere contains about 20 percent of this gas, adequate amounts are provided by maintaining a large volume of atmosphere in tissue culture systems (figure 1). More elaborate systems employ a constant stream of sterile air or gas mixtures.

Experience has shown that tissue cultures often do better if a slight excess of carbon dioxide is present when they are started, and this is provided artificially. Growing cultures of tissues or cells release carbon dioxide. The gas goes into solution readily and is converted to an acid. Excess carbonic acid must be neutralized, and this is readily done by adding bicarbonate or other buffers to the nutrient material.

Environmental Factors

Favorable temperatures must be maintained if tissues are to be successfully

cultured. This is usually provided by a constant-temperature incubator. Warm-blooded vertebrate tissues are incubated at human body temperature (98.6° F.), but tissues from some cold-blooded vertebrates will not tolerate this temperature. Trout tissues, for example, do well at 66.2° F., but fail to grow or even die at slightly higher temperatures (77° F.).

Proper pH, usually within rather narrow limits, is provided by any of several buffer systems which prevent the environment from becoming too acid or too alkaline.

Protection From Disease

As far as micro-organisms are concerned, the glass culture vessels used in tissue culture provide an impenetrable barrier, but the nutrient medium provides a most attractive environment for fungi and bacteria which may fall into it during preparation of the culture. This is an ever-present menace to laboratories, but loss through contamination is reduced by adding small amounts of one or more antibiotics to the nutrient medium.

TYPES OF TISSUE CULTURES

There are several general methods of culturing tissues.

1. Slide cultures represent the least complicated culture system. Small bits of tissue are cultured on a microscope slide cover glass which is held in place over a depression in a small glass or metal plate or in a microscope slide. Since the amount of nutrient is quite small, slide cultures are usually intended for use as short-term cultures. They are, however, the best cultures for microscopic observation. They are easy to set up and can be handled in large numbers.

2. Test tube and flask cultures are larger versions of the slide culture. Small bits of tissue or cells adhere to the surface, either with or without an adhesive clot (figures 1 and 2). Comparatively

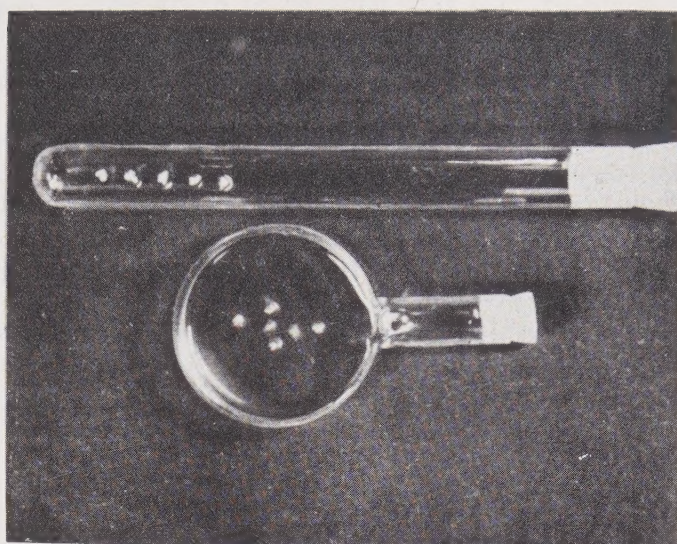


FIGURE 2.-- A tube and Carrel-type flask with bits of tissue embedded in plasma clot. (1/2 size)

large volumes of nutrient surround the tissues, and this is periodically replaced. Flask and tube cultures are probably the most-used form of tissue culture. Their optical characteristics are good but poorer than those of the slide culture. They are easily maintained for long periods of time.

3. Other culture systems. Specialized techniques are employed to maintain viability of cells or bits of tissue in suspension or on special substrates. Viruses are often propagated in such systems, and important biochemical studies are made with growing cell suspensions and other large-scale culture systems.

PREPARATION OF TISSUE CULTURES

The methods by which tissue is prepared for culture vary somewhat with the type of culture, the tissue, and the use for which it is intended. The preparation of simple cultures of trout tissue will serve as a general example.

Healthy fish are selected and quickly killed. External tissues such as gills or fins are removed with sterile instruments and washed with sterile physiological saline. Healthy fish maintained in pure water--trout, for example--are almost free

from external bacteria. The bacteria that were not washed off may be controlled by adding antibiotics to the tissue culture media. For removal of internal organ tissues, the external surface of the fish is thoroughly disinfected. This, of course, destroys some external tissue but does not affect the internal organs. The fish is then opened carefully with sterile instruments, and the desired tissues are removed and placed in cold physiological salt solution. Within a dust-free hood and under aseptic conditions at least as good as in a hospital operating-room, the tissue is cut into small pieces. As the cells are nourished by diffusion, the pieces must be small enough or thin enough that all cells can have access to the nutrients. After blood and cellular debris are washed off, the bits of tissue are placed within tubes or flasks. Some tissues adhere to and grow well on the inside of glass culture vessels, but other tissues must be "glued" in place for best growth to occur. Such tissues are "glued" in place with a thin film of a mixture of chicken plasma and tissue extract. Tissue extracts cause plasma to coagulate in a few minutes, and a clear gelatinlike clot results. After the tissue is in place, the nutrient fluid is added to the vessel. Trout tissues do well in 20 percent serum and 75 percent physiological saline. In order to stimulate growth at maximum rates, 5 percent embryo extract is usually employed in the medium. The chemical identity of the stimulant is unknown; but the effect can be elicited with extracts of embryonic, malignant, or certain adult tissues. The nutrient fluid contains 1,000 units of penicillin and 1,000 micrograms of dihydrostreptomycin per milliliter. This combination of antibiotics keeps most bacteria in check. The addition of a small amount of nontoxic indicator dye permits the investigator to adjust the pH visually and to follow pH changes as they occur.

Several hours after a culture has been started, macrophages or wandering cells appear. These move away from the tissue and spread over the glass or through the clot. This activity is usually followed by a sheetlike growth of epithelium or

epitheliumlike cells (figure 3). As time progresses, spindle cells (the so-called fibroblasts) make their appearance.

When adequate growth has occurred, it may be removed and divided. The portions may then be used to start new cultures. Cells comprising a sheet are also separated from one another--either mechanically or with a digestive enzyme. After being washed, these cells can be divided among additional vessels and used to start new cultures.

APPLICATIONS IN FISH DISEASE RESEARCH

Obviously, the idea in fish tissue culture is to use the preparations or to apply them to an investigation. There is little point in growing tissues or cell sheets for the sake of culture alone; there are much cheaper ways of producing fish flesh.

An immediate but certainly not the only use of tissue culture in fish disease research is its application in virology. Viruses are the tiniest known living particles. Most authorities believe them to be absolute parasites, and they are responsible for many serious diseases of plants and animals. Among fish, a virus has been demonstrated to be the cause of lymphocystis disease (Weissenberg 1951). A serious disease of sockeye salmon is thought to be caused by a virus (Watson 1954), and infectious pancreatic necrosis of trout appears to involve a virus (Snieszko, Wood, and Yasutake, 1957). As far as is known, viruses can reproduce only within living cells. Their presence is often indicated by cellular degeneration. This degeneration or other cellular change can often be demonstrated in tissue culture, and the virus can be transferred from culture to culture without requiring an entire animal or fish. In this way the disease can be diagnosed and the virus identified and propagated. Vaccines can be prepared from viruses grown in tissue cultures, and antibiotics and other chemotherapeutic agents can be screened for their effectiveness in inhibiting or killing viruses.

The process by which host cells engulf and dissolve bacteria, the way in which some bacteria can avoid this fate and instead reproduce within the host cells--these and other aspects of bacterial diseases of fish can be studied in tissue culture systems. Similarly, there are phases of protozoan and helminth infestation which can be studied profitably in cultures of tissue or cells. Physiological and nutritional factors are in themselves often the cause of disease, and they are known to have profound effects on the course of other disease processes. Tissue culture also offers a convenient method for the study of these factors as they apply to fish. The uses to which tissue culture can be applied in fishery research are far more numerous than the personnel available to apply them.

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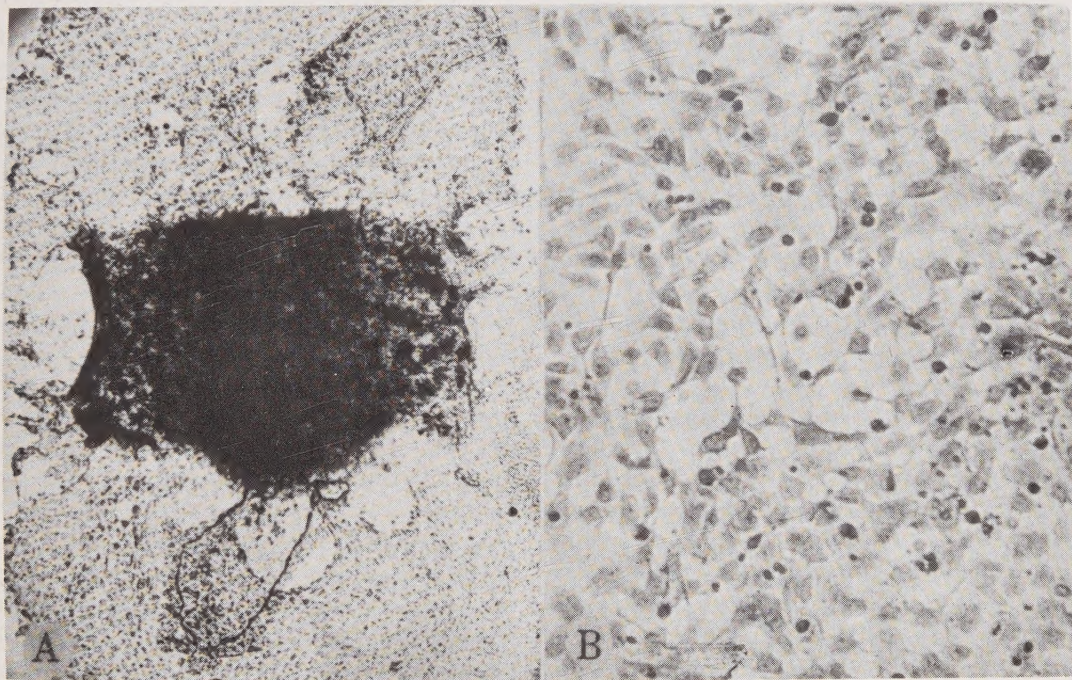


FIGURE 3.-- (A) Sheetlike growth of living cells which have grown out from a bit of tissue during 48 hours' incubation. (X30)
(B) Fixed and stained sheet of trout fin epithelial cells from a 48-hour culture. (250X)

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THE CHEMICAL CONTROL OF SOME DISEASES AND PARASITES OF CHANNEL CATFISH

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THOUGH CONSIDERABLE INFORMATION ON PARASITES AND DISEASES OF TROUT AND SALMON is available, relatively little is known about the diseases of warm-water fishes or the chemical control of these diseases. This was clearly shown in a symposium on fish diseases (Snieszko et al. 1954). The channel catfish (*Ictalurus punctatus*) is a warm-water fish, and disease control has consistently been one of the major problems wherever the hatchery culture of this species has been attempted. Only a small amount of research has been done on this important aspect of the culture of this species.

The logical beginning in a study of possible disease-control methods for the channel catfish was to attempt those methods already known to be successful for other species, cold-water forms included. Before the methods could be tested adequately for effectiveness, it was necessary to know the tolerance of the fish to the chemicals employed. Therefore, in this paper we have reported the limits of tolerance of fingerling channel catfish to

some chemicals beneficial in disease and parasite control, as well as the dosages recommended for such control.

METHODS

To determine the lethal dosages of the chemicals, we used the bioassay method described by Duodoroff (Duodoroff et al. 1951). A series of known concentrations was set up according to the method described by Anderson (1944) and modified for fish by Clemens and Jones (1955). One hundred channel catfish (2 to 3 inches long, 4 to 6 months old) were used, and 10 fish were exposed to each concentration. Each experiment to determine lethal dosages was conducted in the laboratory in University of Oklahoma tapwater at a controlled temperature of 68°, 75°, or 77° F. ($\pm 1^\circ$). As the activity of some chemicals varies with water quality, an analysis of this water is included in table 1. Experiments were run for 96 hours.

Toxicity thresholds are commonly expressed as the median toxicity threshold or the lethal dosage for 50 percent of the fish (i. e., LD₅₀, the dosage at which 50 percent of the experimental fish died in a given period of time). However, the effective use of any chemical to combat disease requires a concentration that is high enough to be effective and low enough to avoid harm to the fish. Therefore, we have included in table 2 the upper and lower limits of tolerance (LD₀ and LD₁₀₀), as well as the LD₅₀, to allow the reader

This investigation was conducted under the Saltonstall-Kennedy Act for commercial fisheries research, University of Oklahoma Research Institute Projects 120 and 132, Contract Nos. 14-19-008-2382 and 14-19-008-2488. Dr. Carl D. Riggs, Project Leader, directed the work.

The authors are entitled to equal credit for this report.

to estimate more accurately the concentrations needed in ponds and troughs or for dips and prolonged treatments, especially for periods of time other than those given in our experiments. All chemical concentrations reported in this paper are based on the whole product as formulated by the manufacturer, not on the basis of active ingredients.

Experiments to determine the efficacy of these chemicals in disease control were conducted in aquaria and in standard hatchery troughs at the U. S. Fish-Cultural Station at Tishomingo, Oklahoma. Chemicals were tested whenever a disease or parasitic infection was found. Controls were used in both aquarium and trough experiments.

We have included in this paper only the diseases for which we have adequate chemical control. All diseases and parasites were diagnosed from living and stained materials, and identifications were made to genus. However, columnaris was identified only in fresh smears and scrapings from infected fish: no cultures or special staining techniques were employed to verify the identification.

RESULTS

Acetic Acid

Acetic acid has been used as a dip at a concentration of 1:500 (2,000 p.p.m.) for 1 minute for the treatment of parasites such as Chilodon, Costia, Trichodina, and gill flukes (Hutchens and Nord 1954). Burrows and Palmer (1949) reported that acetic acid was as good as PMA (pyridylmercuric acetate) for stalked protozoans.

In our experiments the LD₀ for channel catfish was 1,256 p.p.m. (pH 4.5) for 10 minutes, and 629 p.p.m. (pH 4.9) for 1 hour. Treatments lasting 24 hours revealed an LD₀ of 315 p.p.m. (pH 6.1). (See table 2.)

TABLE 1.--Chemical composition of tap water at the University of Oklahoma, Norman, Oklahoma, and the water at the U. S. Fish-Cultural Station, Tishomingo, Oklahoma

[Unless otherwise stated, all values are p.p.m.]

Item	Tap water, University of Oklahoma ^{1/}	Water flowing into hatchery, Tishomingo ^{2/}
Silica (SiO ₂)-----	12	-----
Iron (Fe)-----	.02	-----
Calcium (Ca)-----	4.0	54.0
Magnesium (Mg)-----	2.8	-----
Sodium (Na)-----	127.0	5.3
Potassium (K)-----	1.1	1.5
Carbonate (CaCO ₃)-----	16.0	60
Bicarbonate (CaCO ₃)----	310	285
Sulfate (SO ₄)-----	15	7.2
Chloride (Cl)-----	4.8	15.0
Fluoride (F)-----	.1	-----
Nitrate (NO ₃)-----	1.4	-----
Dissolved solids-----	336	-----
Total hardness (CaCO ₃)	22	-----
Specific conductance at 25° C. ----(microhms)	554	552
pH-----	8.7	8.4
Color units-----	5.0	-----
Percentage sodium ^{3/} ----	92.0	-----
Phosphorus (P)-----	-----	.04

^{1/}Analysis made by the U. S. Geological Survey, Stillwater, Oklahoma.

^{2/}Analysis made by the authors.

^{3/}Percentage of cations.

Our laboratory data suggest that channel catfish could withstand a 1:500 concentration for 1 minute, but our field tests indicate that a dip of 45 seconds was less harmful to the fish. Two or three dips effectively controlled, but did not eliminate, Colponema (sp.).^{1/} (See table 3.) Fish with Costia, Trichodina, and flukes are treated effectively with other chemicals in a prolonged dip, and we feel that by using these chemicals we have a safer treatment.

^{1/}The parasite is very similar to Costia but occurs on the gills only and is resistant to pyridylmercuric acetate (treatment with 8 p.p.m. for 2 hours required for control). The identification of Colponema is tentative.

TABLE 2.--Limits of tolerance of fingerling channel catfish in some chemicals beneficial in disease control

Chemical	Temperature, Fahrenheit	Lethal dosage	Lethal dosages in p.p.m. ^{1/} at various exposure times in hours									
			1	2	4	8	24	48	72	96		
Pyridylmercuric acetate, "PMA" ^{2/} 80% active	75°	LD ₁₀₀	50.0	50.0	21.0	6.7	5.0	0.89	0.50	-----		
		LD ₅₀	-----	34.0	11.9	5.8	3.8	-----	.49	-----		
		LD ₀	37.6	28.0	8.9	5.0	2.8	.37	.37	-----		
Trypaflavine (acriflavine, neutral) ^{3/}	68°	LD ₁₀₀	787.5	56.0	42.0	-----	17.9	10.0	10.0	7.5		
		LD ₅₀	87.5	42.4	27.6	-----	11.5	6.8	6.4	4.9		
		LD ₀	56.0	24.0	18.0	-----	7.5	4.2	4.2	3.2		
Acetic acid	77°	LD ₁₀₀	1,256	629	629	629	629	629	629	-----		
		LD ₅₀	889	446	446	446	446	388	270	270		
		LD ₀	629	315	315	315	315	158	158	-----		
Malachite green, oxalate salt ^{4/}	77°	LD ₁₀₀	1.00	0.50	0.25	0.25	0.19	0.19	0.19	0.19		
		LD ₅₀	.79	.31	.20	.17	.14	.14	.14	.14		
		LD ₀	.40	.25	.16	.10	.10	.10	.10	.10		
Formalin	77°	LD ₁₀₀	> 500	500	316	199	126	126	126	126		
		LD ₅₀	500	263	165	138	87	69	69	69		
		LD ₀	316	126	126	79	50	50	50	50		
Phenylmercuric acetate, "Tag" ^{5/} 10% solution	68°	LD ₁₀₀	> 40.0	16.90	5.30	4.00	2.30	1.30	1.30	0.95		
		LD ₅₀	> 40.0	8.30	2.70	2.30	1.46	.78	.60	.58		
		LD ₀	> 40.0	3.00	1.30	1.30	.95	.40	.40	.40		

^{1/}Concentrations in p.p.m. are based on the whole product as furnished by the manufacturer, not on active ingredients.

^{2/}Mallinckrodt Chemical Works, St. Louis, Mo.

^{3/}Bios Laboratory, New York.

^{4/}Fisher Scientific Company, New York.

^{5/}California Spray-Chemical Corporation, Richmond, California.

TABLE 3.--The recommended chemical treatment for some diseases and parasites of channel catfish

Disease or parasite	Chemical	Treatment	Remarks
<u>Ichthyophthirius</u> sp.	Pyridylmercuric acetate, PMA	2 p.p.m. for 1 hour daily for duration of life cycle. Completely flush all the chemical after each treatment.	Treatments must begin before infection is severe. Duration of life cycle depends on temperature: at 75° F., treatments should continue 10 days; at 50° F., treatments should continue 30 days or longer.
<u>Colponema</u> sp.	Phenylmercuric acetate, Tag Trypaflavine (acriflavine, neutral) Acetic acid, glacial, CP grade	2 p.p.m. for 1 hour. 4 p.p.m. for 48 hours. 1:500 dip for 45 seconds.	One treatment sufficient. Pyridylmercuric acetate at 8 p.p.m. for 2 hours at 62° F. also effective. One treatment sufficient. Two or three dips 48 hours apart necessary for control. Duration of dip very critical.
Gill diseases	Pyridylmercuric acetate, PMA	2 p.p.m. for 1 hour.	Treat as often as necessary. Apparently eliminates columnaris infections and reduces mortalities due to dietary gill diseases.
<u>Trichodina</u> sp.	Pyridylmercuric acetate, PMA	2 p.p.m. for 1 hour.	One treatment sufficient, but reinfection often occurs.
<u>Costia</u> sp.	Pyridylmercuric acetate, PMA	2 p.p.m. for 1 hour.	One treatment sufficient, but reinfection often occurs.
<u>Scyphidia</u> sp.	Trypaflavine. Phenylmercuric acetate, Tag	2 p.p.m. for 24 hours. 2 p.p.m. for 1 hour.	As good as Tag but more expensive; probably safer treatment than Tag. One treatment sufficient. Duration of treatment should not exceed 1 hour, or delayed mortality may occur.
<u>Dactylogyrus</u> sp.	Formalin	1:4,000 for 1 hour.	May adversely affect fish at temperatures above 79° F. Use only fresh, clear formaldehyde.
<u>Saprolegnia</u> sp.	Malachite green, oxalate salt	1 p.p.m. as a flush treatment	Flush trough so that chemical is entirely removed in 1 hour; repeat as often as needed. Effective for fish or eggs.

Formalin

Formalin in concentrations of 1:4,000 has been used as a 1-hour treatment for Argulus, Chilodon, Costia, fin rot, flukes, Ichthyophthirius, Salmincola, and Trichodina (Hutchens and Nord 1954). Burrows (1949) stated that a 1-hour treatment with 1:4,000 formalin was effective on external parasites except Ichthyophthirius, parasitic copepods, and Saprolegnia. Eicher (1947) recommended a treatment with 1:2,000 for 1 hour followed by a flush treatment with malachite green as effective for fin rot. Eicher also stated that formalin was an effective prophylactic treatment for Saprolegnia parasitica on salmon eggs.

The LD₀ of formalin for channel catfish was 316 p.p.m. (1:3,200) for 1 hour, 126 p.p.m. for 2 hours, 126 p.p.m. for 4 hours, 79 p.p.m. for 8 hours, 50 p.p.m. for 24 hours, and 50 p.p.m. for 96 hours (table 2).

Formalin was used a number of times on channel catfish for external parasites during the course of our investigations. We found it effective at 1:4,000 for 1 hour for the removal of Dactylogyrus (gill flukes), Scyphidia, Trichodina (table 3), and to a lesser extent for removal of leeches. However, in treatments with 1:4,000 formalin, either on juveniles or on adults, the fish appeared harmed, as they "topped," became sluggish, and lost their appetite. At temperatures about 75° F., there was some mortality that was attributed to the chemical. For Costia, Ichthyophthirius, Trichodina, Scyphidia, Saprolegnia, and parasitic copepods, other treatments are considered better and easier on the fish.

Malachite Green

Malachite green is often used as a fungicide for fish eggs, and the following doses have been recommended for Saprolegnia: 1:200,000 (5 p.p.m.) for 1 hour as a prophylactic treatment (Burrows 1949), 1:15,000 (66 p.p.m.) for a 30-second dip (O'Donnell 1941), and 1:180,000 for 45 minutes as a pond treatment (Allison 1954).

Eicher (1947) used a flush treatment with malachite green after a formalin treatment to control fin rot in rainbow trout fry.

The LD₀ of channel catfish treated with malachite green (oxalate salt) was greater than 2 p.p.m. for 30 minutes, 0.4 p.p.m. for 1 hour, 0.25 p.p.m. for 2 hours, 0.16 p.p.m. for 4 hours, 0.10 p.p.m. for 8 hours, 0.10 p.p.m. for as much as 96 hours (table 2).

A flush treatment with 1 p.p.m., progressively diluted by inflowing water so that all the chemical was washed from the trough within about 1 hour, was administered to adult fish on alternate days for a month with no apparent harm and was used every day for a week without any detrimental effects on fingerling catfish reared in troughs. It proved effective in the control of Saprolegnia on both the fish and the eggs (table 3). The dilution should begin immediately upon application of the chemical, as an exposure for 1 hour to 0.79 p.p.m. is 50 percent lethal. The progressive dilution in this manner leaves some margin of safety, and the treatment is well tolerated by the fish and is convenient to the fish culturist.

Pyridylmercuric Acetate

Pyridylmercuric acetate (PMA) has proved effective in the control of gill diseases (Rucker 1948, Rucker and Whipple 1951, Rodgers et al. 1951, Snieszko 1949, and Van Horn and Katz 1946). Burrows and Palmer (1949) showed that blueback salmon withstood 12 consecutive weekly treatments with PMA at 1:50,000 for 1 hour. Katz (1948) determined that 0.5 p.p.m. was the minimum lethal dosage for Notropis atherinoides for 5 days, that PMA was an efficient slime mold agent, and that it effectively controlled external protozoan parasites.

The LD₀ of pyridylmercuric acetate for fingerling channel catfish at 75° F. was 37.6 p.p.m. for 1 hour, 28.0 p.p.m. for 2 hours, 8.9 p.p.m. for 4 hours, 5.0 p.p.m. for 8 hours, 2.8 p.p.m. for 24 hours, and 0.37 p.p.m. for 72 hours (table 2).

We treated channel catfish of all ages ranging from 1 week to adulthood with concentrations of PMA up to 2 p.p.m. for 1 hour and found it particularly effective for columnaris diseases on skin or gills and Trichodina (table 3), but not effective for gill flukes or leeches; at least, single, infrequent treatments did not affect the latter two groups of parasites. Channel catfish can tolerate daily treatments of this chemical for periods exceeding 6 weeks. Fish which were treated daily did not lose their appetite and they grew as well as the controls. Present information indicates that treatments with 2 p.p.m. for 1 hour on alternate days will effectively control all the protozoan parasites (except Colponema, which required treatment with 8 p.p.m. for 2 hours) which were found infecting catfish at Tishomingo. However, single, infrequent treatments did not eliminate Scyphidia or Ichthyophthirius; whereas daily medication effectively controlled these parasites (table 3).

We have one report from another hatchery which suggests that PMA may be toxic to channel catfish under some conditions. Recently, Allison (1957) has shown that certain lots of PMA are toxic to rainbow trout. At the moment it appears that the toxic elements are contained in lots in which the active ingredients are high (87 percent). Therefore, each lot of PMA should be tested to determine its toxicity before large-scale application is made. Observations on the tests should be made for at least 24 hours, as the mortalities from treatments with pyridylmercuric acetate are delayed.

Phenylmercuric Acetate

Because pyridylmercuric acetate was so beneficial, another mercurial compound, phenylmercuric acetate (known as Tag), was tested in the laboratory to determine the lethal dosages for fingerling channel catfish. The LD₀ at 68° F. was greater than 40 p.p.m. for 1 hour, 3.0 p.p.m. for 2 hours, 1.3 p.p.m. for 4 hours, 0.95 p.p.m. for 24 hours, 0.4 p.p.m. for 48 hours, and 0.4 p.p.m. for periods up to 96 hours (table 2).

Field tests indicated that Tag at a concentration of 2 p.p.m. for 1 hour at 50° F. eliminated Scyphidia from channel catfish gills (table 3). Aquarium tests showed that fish treated with 2 p.p.m. for 2 hours at 60° F. died 2 days later, but as much as 10 p.p.m. for 1 hour did not cause delayed mortality. Seventeen thousand fish which were treated with 2 p.p.m. for 1 hour at 50° F. were free of Scyphidia, and no mortality occurred.

Limited observations suggest that it is not effective against gill flukes but may kill other protozoans. This chemical, at a concentration of 2 p.p.m. for 1 hour at 62° F., completely eliminates Colponema; whereas 8 p.p.m. of PMA for 2 hours at 62° F. was required to eliminate this organism.

Trypaflavine (Acriflavine, Neutral)

Blake (1931) used a 1:2,000 solution of acriflavine to disinfect fish eggs. The value of this chemical was verified and its use extended by Atkinson (1932), Gee and Sarles (1942), and O'Donnell (1947). Schäperclaus (1954) recommended a bath of 10 p.p.m. trypaflavine for the treatment of Ichthyophthirius on guppies.

Laboratory bioassays showed that the LD₀ concentrations of this chemical for channel catfish at 68° F. were as follows: 56.0 p.p.m. for 1 hour, 24.0 p.p.m. for 2 hours, 18.0 p.p.m. for 4 hours, 7.5 p.p.m. for 24 hours, 4.2 p.p.m. for 48 hours, 4.2 p.p.m. for 72 hours, and 3.2 p.p.m. for 96 hours (table 2).

The effectiveness of this chemical on Colponema and Scyphidia was tested at a water temperature of 50° F. Experiments to determine the relative value of acetic acid and trypaflavine on Colponema revealed that trypaflavine completely eliminated the parasite when used at 4 p.p.m. for 48 hours, whereas 2 to 3 dips of acetic acid were required for effective control.

A concentration of 2 p.p.m. for 24 hours effectively removed Scyphidia, and this treatment was judged to be safer than any other chemical for the treatment of this parasite (table 3), at least until additional information can be obtained on Tag.

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CHEAP TRANSPORTATION FOR SMALL QUANTITIES OF FINGERLINGS

A small wooden box made it possible for Francis H. Bezdek of Lisbon, Ohio, to transport 2- to 3-inch fathead (*Pimephales promelas*) and bluntnose (*Hyborhynchus notatus*) minnows for as long as 6 hours.

The box was made of 3/4-inch redwood or exterior-type plywood. Screw-nails were used in its assembly, melted paraffin was used to seal joints, and a small turn-buckle was put on each end to counteract outward water pressure. The box had 2 end compartments (each about 9 inches high by 10 inches wide by 18 inches long) and a screened center compartment (about 9 by 10 by 6 inches long). Perforated 1/4-inch copper tubing bent in a U and stapled to the bottom provided aeration. If the air bubbles from the perforations were too large, finely woven cloth was wrapped around and sealed to the tubing with wax or other substance. The end compartments were for fish; the center was for ice when water temperatures neared 60° F.

Rubber tubing (1/4 inch) carried oxygen from a tank to the copper tubing (extended outside the live cars near the top and above the normal water line). As live cars were never more than half full, expansion of the compressed oxygen helped cool the water.

Lids were flush with the tops of the boxes, held in place beneath by a narrow wooden strip around the edge of each compartment and above by four door buttons.

"A live car and an oxygen tank (with attached low-pressure gauge) were put in the back of a small foreign car," writes Mr. Bezdek. "The live car was half filled with water, and 2,000 to 3,000 fingerlings were put in each end compartment. For easier handling, about 500 fish (2 pounds) were put in perforated plastic bags. Use of bags appeared to reduce injury from bruising in transit. Oxygen was bubbled through the live car only when fish showed distress. When in bags, however, the fish, through continued efforts to escape, tended to boil and aerate the water."



(Photograph courtesy of Mr. Bezdek)

THE USE OF COPPER SULFATE TO INCREASE FYKE-NET CATCHES

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COPPER SULFATE was first used as a fish poison in 1914, and since then it has been used periodically in this capacity. Copper sulfate has also been in general use as an algaecide for the past 50 years. In 1953 it was used experimentally by the Massachusetts Division of Fisheries and Game in accelerating fish movements in ponds which were being fyke-trapped for the removal of overabundant pan and weed species; it has since been used as a standard accessory tool in netting operations.

The merit of this compound as an aid to increase fyke-net catches was demonstrated during August 1952 in Lake Quannopowitt, Wakefield, Massachusetts, while a Pond Management Unit of the Bureau of Wildlife Research and Management was engaged in a thinning operation. The unit had set 6 fyke nets in this body of water on August 12, and by August 21 the catch per net day had declined from 148 pounds to 11 pounds. On this latter date personnel employed by the town water commission applied copper sulfate to the pond, as an algaecide, at a concentration of 1.0 p. p. m. The following days' lifts contained 173 pounds of fish per net, the highest 24-hour return recorded during the operation. From this peak the catch declined to 15 pounds per net day on the 28th, when operations were concluded. This application of copper sulfate apparently produced an increased total harvest of 36 percent to 45 percent in this instance, depending on the method of extrapolating pretreatment catches into problematical posttreatment catches in the

absence of the copper sulfate application (figure 1).

Of greater significance than the increase in total catch were the increases observed in the harvest of various species. White perch formed 56.8 percent (or 2,967 pounds) by weight of the total overall catch. Of the latter figure, 607 pounds (or 20 percent) was taken after the application of copper sulfate. Yellow perch made up 8.0 percent (or 424 pounds) of the catch. Of the latter, 63 percent (or 269 pounds) was taken after the treatment. Black crappie formed 11.0 percent (or 584 pounds) of the total weight. Of this total, 550 pounds (or 94.1 percent) was taken after the copper sulfate had been applied.

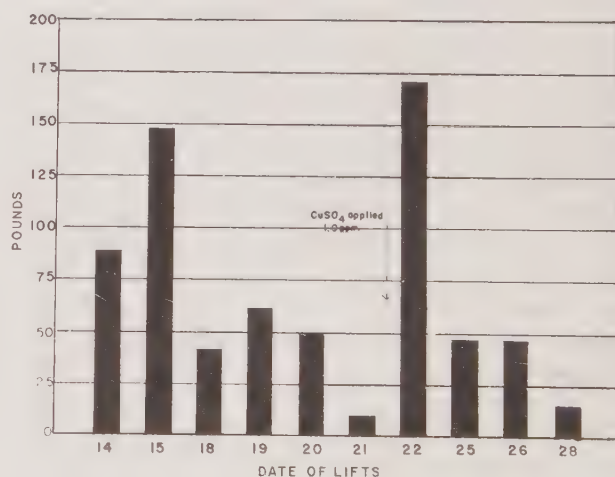


FIGURE 1.--Catch in pounds per net day in Lake Quannopowitt, Wakefield, August 14 to 28, 1952.

Bluegill and pumpkinseed made up 14.9 percent (or 787 pounds) of the total catch. Of this latter group, 88.0 percent (or 693 pounds) was harvested after the introduction of copper sulfate. The remaining 10 percent of the total catch was composed of carp, eels, golden shiners, smallmouth bass, and pickerel--all of which were taken in increased numbers after the application of copper sulfate.

These very large and easily observed increased catches led to speculations concerning the practicability of using low concentrations of this compound as a possible nontoxic irritant to increase summer net catches.

Effects of Copper Sulfate on Fish

One very important consideration in the use of copper sulfate as a fish irritant is the effect of this substance on fish production. It is a more or less accepted fact that copper sulfate kills fish by a combination of chemical and physical injuries rather than through true toxic action, as pointed out by Ellis (1937). The salts of the heavy metals combine readily with the mucus that is secreted by the skin, mouth, and gills, forming insoluble compounds. If the concentration of the salt is high enough, the precipitated compound covers the gills and death results from respiratory failure. If the concentration of the copper sulfate is below the lethal level, the secretion of additional mucus will wash away the precipitated compound as fast as it is formed and before serious damage to the fish results. It is conjectured, then, that increased fish movements in low concentrations of copper salts are the result of the irritant action of the precipitate.

Because of the widespread use of this compound in aquatic situations over the past 5 years, a great number of references to its toxicity to fish can be found. Unfortunately, however, the concentrations necessary to kill fish depend on the character of the water in question: its temperature, hardness, the amount of organic matter present, and other factors. In view of the fact that Lake Quannopowitt

had been treated almost annually since 1927 with concentrations ranging between 0.12 and 1.0 p. p. m. with no recorded fish kills, and inasmuch as this body of water is fairly typical in its pH and total hardness ranges of the characteristically soft waters of Massachusetts, it was assumed that immediate toxicity for fish would not be a problem if concentrations were kept at 0.5 p. p. m. or less.

A second consideration is the possible long-range effects of applications of copper sulfate with regard to the productivity of any given body of water. Despite a long history of copper sulfate treatment, net catches indicate that Lake Quannopowitt is above average for Massachusetts in productivity. There can be found no definite account in which a decline in fishing has been shown to be the result of the continued use of copper sulfate, but Moyle (1949) has pointed out that average commercial fishing yields per acre over a 24-year period were actually lower for a group of 5 untreated lakes than for 4 adjacent treated lakes in southern Minnesota, and that fish harvest by anglers from 2 treated lakes was higher during a summer creel-census period than the average for all Minnesota lakes during the same period. It was concluded that there was no evidence to indicate that the applications would be harmful to the fish population involved.

Experimental Use of Copper Sulfate

During the summer of 1953, 4 ponds ranging in size from 21 to 52 acres were selected for experimental use in determining the practicability of copper sulfate applications as an aid in increasing fyke-net catches. The data collected at Lake Quannopowitt had given no indication of the potential with regard to bullhead populations, the effectiveness of copper sulfate at concentrations less than 1.0 p. p. m., the results that might be obtained from spraying only areas adjacent to the traps, or the effects of repeated applications of minimal concentrations. This information was collected during 1953 on the 4 experimental ponds.

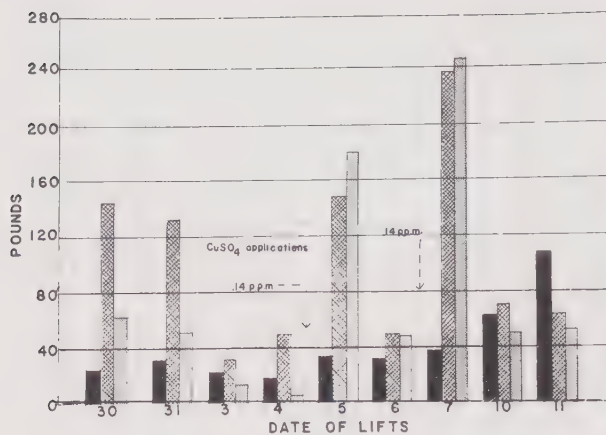


FIGURE 2.--Catch in pounds per day of yellow perch (solid rectangles), sunfishes (cross-hatched), and white perch (shaded) in Jordan Pond, Shrewsbury, July 30 to August 11, 1953.

Jordan Pond, Shrewsbury. -- This 21-acre pond carried large populations of bluegill and pumpkinseed (hereafter referred to collectively as sunfishes), white perch, and yellow perch. Four fyke nets with 200-foot shore-tied heart leads were set on July 29 and fished in the same locations until August 11. On August 4, 54 pounds of copper sulfate, equal to 0.14 p.p.m., was sprayed over the entire surface of the pond. The pond was sprayed again on August 6 with a similar amount, which gave a cumulative total of 0.28 p.p.m. During this period, surface water temperatures ranged from 70° to 75° F. The species tabulated in figure 2 represent 94.6 percent by weight of the total catch. Other species taken, but too sparsely represented for valid comparisons, included white suckers, golden shiners, black crappie, bullheads, chain pickerel, and largemouth bass.

Duck Pond, Groton. -- This pond covers 52 acres and has a mean depth of 6 feet. It has extensive areas of aquatic weed growth and supports a population that is composed principally of bullheads, chubsuckers, and yellow perch. Six fyke nets were set in the pond on July 15 and fished, without being moved, until July 28. On July 20, 43 pounds of copper sulfate (or

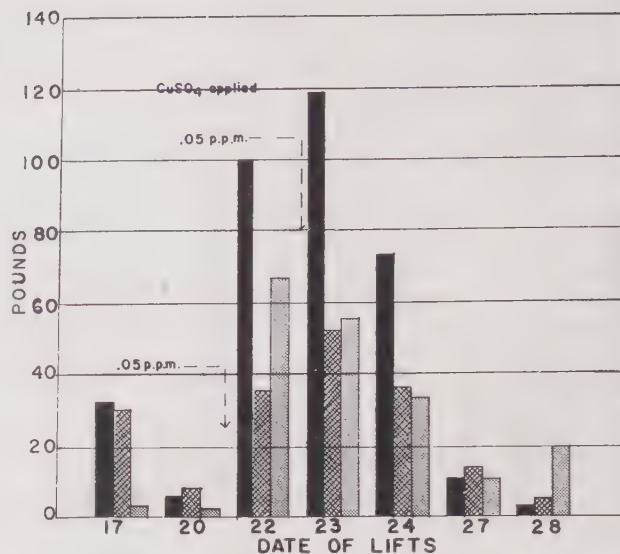


FIGURE 3.--Catch in pounds per day of brown bullheads (solid rectangles), chubsuckers (the cross-hatched rectangles), and yellow perch (shaded) in Duck Pond, Groton, July 15 to 28, 1953.

0.05 p.p.m.) was sprayed over the areas adjacent to the sets. On July 22 this procedure was repeated, so that there was a cumulative total of 0.1 p.p.m. During the netting operations surface water temperatures ranged between 72° and 77° F. Results for the 3 predominant species are given in figure 3.

Massapoag Pond, Lunenburg. -- Seven fyke nets were set in this 38-acre pond on July 1 and fished, in the same spots, until July 11. On July 7, 34 pounds of copper sulfate (or 0.025 p.p.m.) was applied in a band 50 feet wide around the margin of the pond. On July 8 and July 9 this procedure was again followed, but 68 pounds of the compound was used on these occasions, giving a cumulative concentration of 0.125 p.p.m. Sunfishes, brown and yellow bullheads, and yellow perch made up 86 percent of the total weight of the catch (figure 4). Surface temperatures during this period ranged from 74° to 77° F.

Wedge Pond, Winchester. -- Five fyke nets were set in this 21-acre pond June 25 and fished, without moving the sets, until July 14. Eighty-two pounds of copper

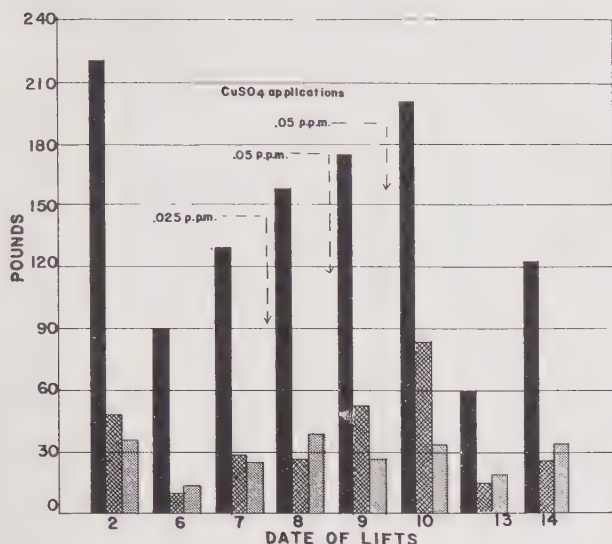


FIGURE 4.--Catch in pounds per day of sunfishes (solid rectangles), bullheads (cross-hatched), and yellow perch (shaded) in Massapoag Pond, Lunenburg, July 1 to 14, 1953.

sulfate was sprayed over the entire surface of the pond on July 7, giving a concentration of 0.20 p.p.m. This was repeated on July 9, so that the pond received a cumulative total of 0.40 p.p.m. Surface water temperatures ranged from 76° to 80° F. during this period. Sunfishes made up 81 percent of the total weight of the catch (figure 5). The remainder of the weight was composed of chubsuckers, white suckers, golden shiners, black crappie, yellow perch, brown bullheads, chain pickerel, and largemouth bass.

The work on Jordan, Duck, Massapoag, and Wedge Ponds during the summer of 1953 proved the practicability of using copper sulfate to increase fyke-net catches in Massachusetts, even at extremely low concentrations. In Wedge Pond (figure 5), an application of 0.10 p.p.m. increased the poundage taken 4.5 times over that of the previous 24-hour period. In Duck Pond, an application of 0.05 p.p.m. resulted in a 12-fold increase in the poundage harvested (figure 3) and demonstrated its potential with regard to bullhead populations. In both Jordan Pond

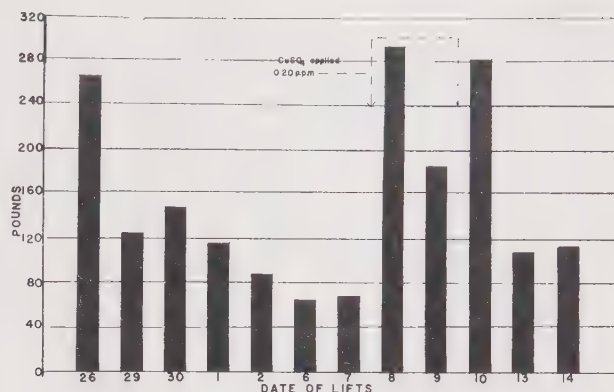


FIGURE 5.--Catch in pounds per day of sunfishes in Wedge Pond, Winchester, June 26 to July 14, 1953.

(Drawings courtesy of the authors)

and Wedge Pond, more than 125 pounds per acre was removed during the netting period.^{1/}

A rapid drop in netting success takes place about 24 hours after the application of the copper sulfate, regardless of the concentration used. Apparently this lag can be overcome by applying minimal concentrations on successive days (figure 4).

All species of fish encountered showed a definite response to the treatment, and the degree of netting success for different species appears to be determined solely by their availability rather than influenced by differential responses to the copper sulfate. Spraying the entire surface of the pond or all areas adjacent to the nets seemed to give the best results. Marginal spraying (figure 4) did not increase, relatively, the catch of sunfishes over other species, as was hoped.

Management Applications

Copper sulfate is now employed in Massachusetts as a more or less routine

^{1/}The carrying capacities of Massachusetts waters have been determined, by measurements made to date on 42 reclaimed ponds totaling 1,432 acres, to range from 17 to 280 pounds per acre. Ninety-five percent of these ponds carried 30 to 140 pounds per acre.

accessory to netting operations. Pond Management Units are now equipped with gasoline-operated centrifugal pumps, and the chemical is dispersed in an aqueous solution with spray booms developed for the application of rotenone. Currently, a concentration of 0.15 p.p.m. is being used. Experiences to date have paralleled those gained in 1953, and in all instances the use of the compound has materially increased the total catches.

The time consumed in the mixing and application of copper sulfate is insignificant, as is the cost, in view of the results. The commercial grade of powdered copper sulfate used during 1953 and 1954 was purchased for 10.7 cents a pound. At a concentration of 0.15 p.p.m., the cost of the material amounted to 4 cents per acre-foot.

Although copper sulfate has been used only in connection with fyke netting, the chemical would seem to have possibilities in other facets of fishery work. Its use in connection with gill nets in routine lake and pond surveys would undoubtedly facilitate larger collections in shorter periods of time. Its effectiveness in moving sunfishes might well be applicable to bluegill trapping operations in farm-pond management, especially as it can be used at concentrations below the demonstrated lethal

limits for most plankters. It could be of value in making population estimates when large collections of fish taken over short periods of time are necessary or desirable. In Massachusetts its use has more than doubled the efficiency of fish trapping operations and greatly reduced the time and costs involved.

Acknowledgments

The authors wish to thank Messrs. Harold Bitzer, Carleton Hudson, and Michael Miskewich for their part in the field work, and Mr. Richard H. Stroud, who planned the work done in 1953.

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SODIUM FLUORECEIN AND THE TOXICITY OF PRONOXFISH

Fishery workers often use a dye to mark waters treated with fish toxicants. Charles L. Sowards, fishery biologist of the Wyoming Game and Fish Commission, has recently reported on a test made to find out whether use of sodium fluorecein would affect the toxicity of Pronoxfish.

Six small longnose dace (*Rhinichthys cataractae ocella*) were put in each of three 12-liter aquaria containing identical creek water. The first aquarium then received 0.0063 milliliter of Pronoxfish (the same concentration as 1 gallon per 6 acre-feet); the second, 0.0063 milliliter of Pronoxfish

and enough sodium fluorecein to color the water bright green; the third aquarium was the control.

"The effect of Pronoxfish on longnose dace was not altered by the sodium fluorecein," writes Mr. Sowards. "The times of discomfort, distress, and death were almost identical in the first two aquaria. The control fish seemed comfortable and healthy 38 hours after the others had died.

"Apparently sodium fluorecein does not harm longnose dace, as 6 seemed comfortable and healthy after living in a brilliant green solution for a little over 5 days."

STOCK THOSE LAKES BY AIR

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DO YOU HAPPEN TO HAVE A FAVORITE LAKE that needs to have some heavy stocking? If so, it may be feasible for you to try air transportation and release on a large scale.

Spirit Lake, which lies at the foot of Mount St. Helens in the State of Washington, was the primary target for an air drop of about 200,000 sockeye salmon (*Oncorhynchus nerka*), released from the air during the fall of 1956.

Undesirable extra handling or long periods of transportation prompted the search for a rapid and practical means of accomplishing this stocking program. Aerial stocking has been an accepted tool of fishery management in completing such projects for many years. The problem in this situation, however, was to develop acceptable facilities and methods for transporting and dropping large quantities of fish at one time. Approximately 2.5 tons of fish and 24 tons of water were loaded, transported, and dropped from the air before the project was finished.

This undertaking was regarded strictly as a biological project, but the circumstances of its successful completion are of interest and paramount importance. A large plane capable of carrying a payload of approximately 5 tons was the first requisite. This requirement was met by negotiation with the U. S. Air Force Reserve. Interagency cooperation resulted in the satisfactory completion of the stocking program for the Service and served as a training mission for the reservists.

The two important factors, economy and light weight, had to be considered in the design and construction of a tank which

would fit in the C-46-type aircraft and hold the desired number of fish. The tank, as completed, was in the shape of a cylinder 14 feet long by 3.5 feet in diameter and held approximately 1,000 gallons of water. A circular hatch, 2 feet in diameter, was located on the upper side, at one end of the cylinder. A pipe, 2 feet in diameter and approximately 10 feet long, led from one end of the tank to the side of the aircraft. The details of design and construction are shown in figures 1-A and 1-B.

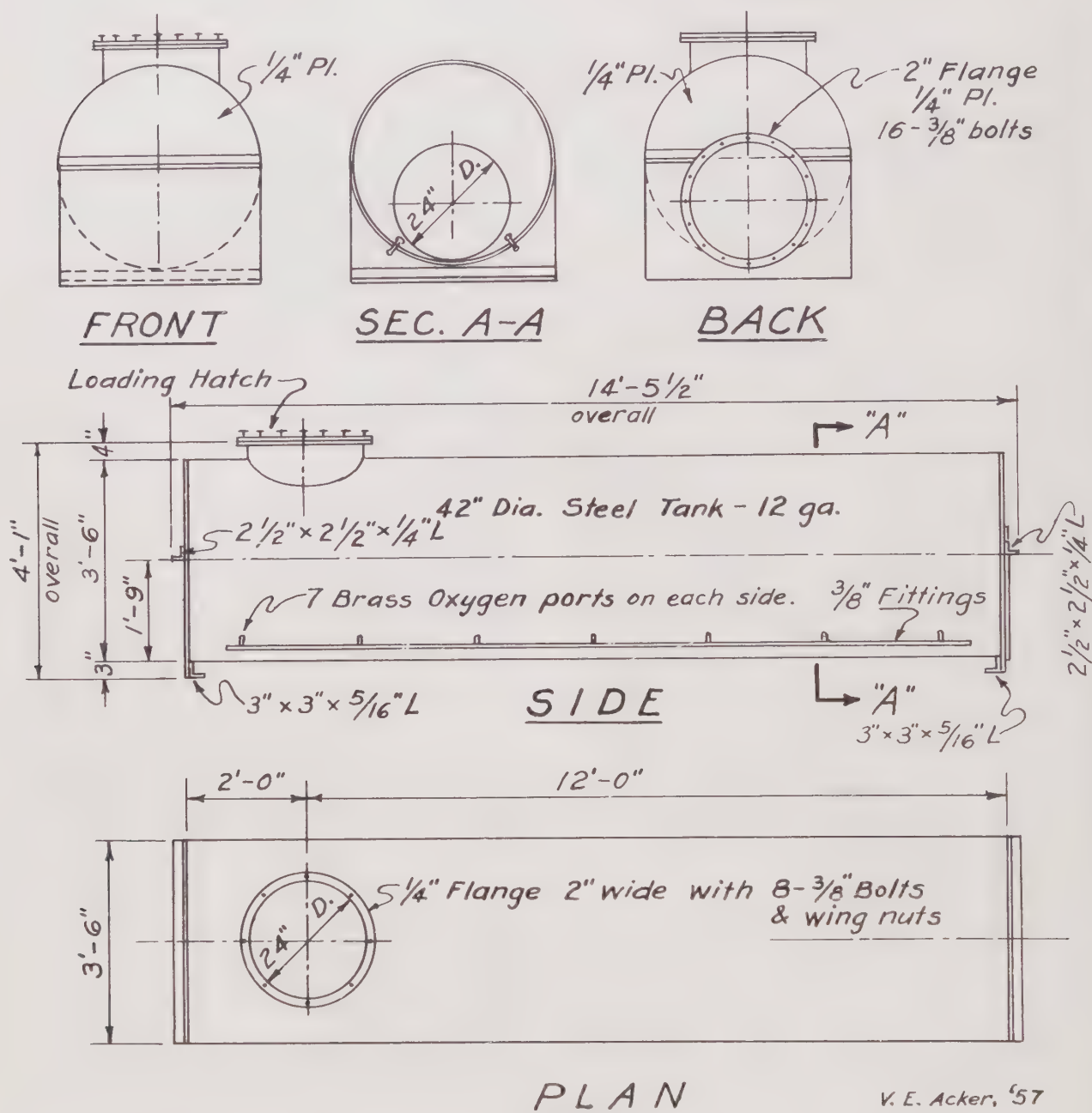
Copper tubing and brass fittings in conjunction with air stones were attached for dispersing oxygen throughout the tank while fish were present. There were 14 air stones in all, with 7 placed on each side near the bottom of the tank (figure 2).

The "drop speed" over the lake (120 miles per hour) and the size of the lake allowed approximately 40 seconds over the water. Consequently, a light-weight apparatus which would release approximately 1,000 gallons of water and 1,000 pounds of fish within this time was needed. Conventional snap-release valves of the size required (24 inches in diameter) were too heavy and also expensive. Considerable thought and ingenuity resulted in a satisfactory release method which would function within the maximum desired time of 5 seconds.

The mechanism used for a rapid release of water was constructed of a piece of rubberized canvas approximately 3 feet wide and 7 feet long. The canvas was vulcanized after being rolled into a tube (2 feet in diameter by 3 feet long) which was open at both ends. One end of the

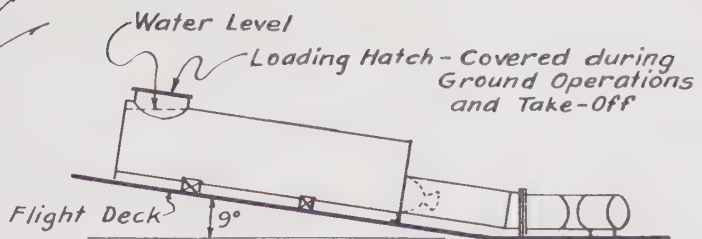
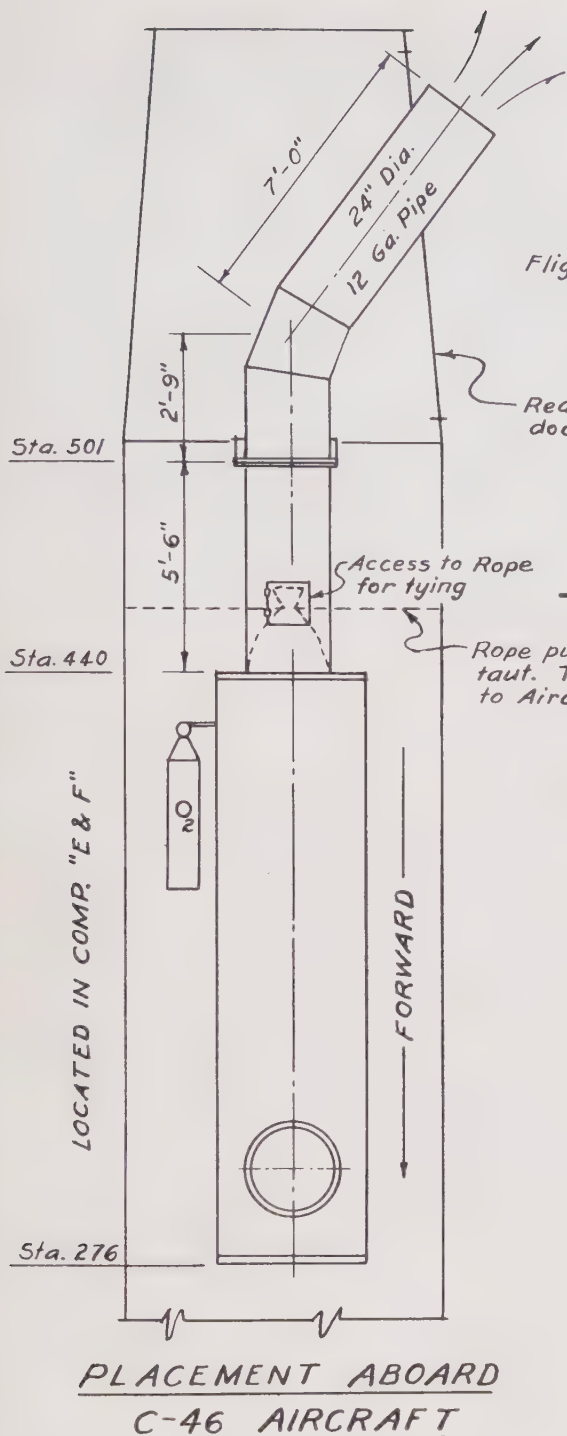
tube was fitted to the outlet of the tank inside the outlet pipe (2 feet in diameter). The other end was crimped in a manner similar to that used in closing a paper bag. This crimped end was secured by means of a simple loop of a double line of 3/8-inch manila rope. The ends of the

double line of rope were pulled through holes in the side of the 2-foot pipe and tightly secured outside the pipe. The release mechanism is shown ready for use in figure 3; the tank overflow tube is taken out when the fish are dropped.

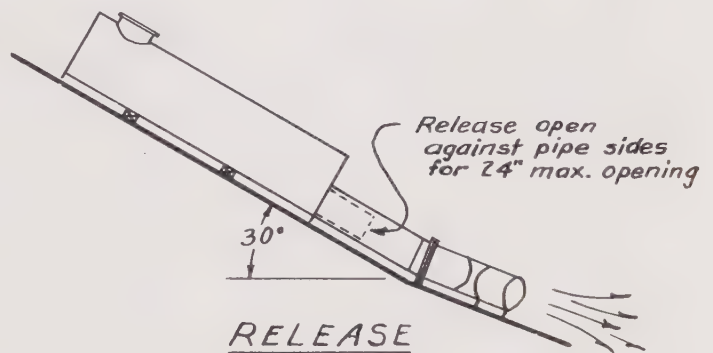
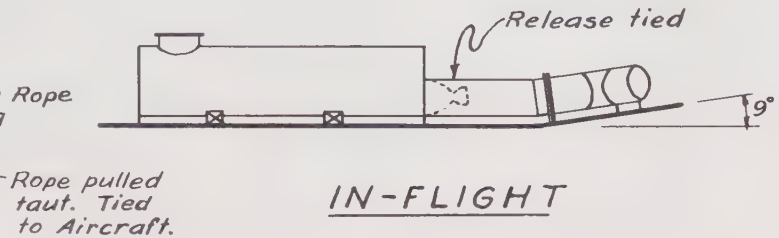


V. E. Acker, '57

FIGURE 1-A.--The 1,000-gallon air transportation tank: a quick release for air drops of fish, adapted for use in C-46 aircraft.



LOADING ON GROUND



ATTITUDES OF TANK ON GROUND AND IN AIR



V. E. Acker, '57

FIGURE 1-B.--The 1,000-gallon air transportation tank: a quick release for air drops of fish, adapted for use in C-46 aircraft.

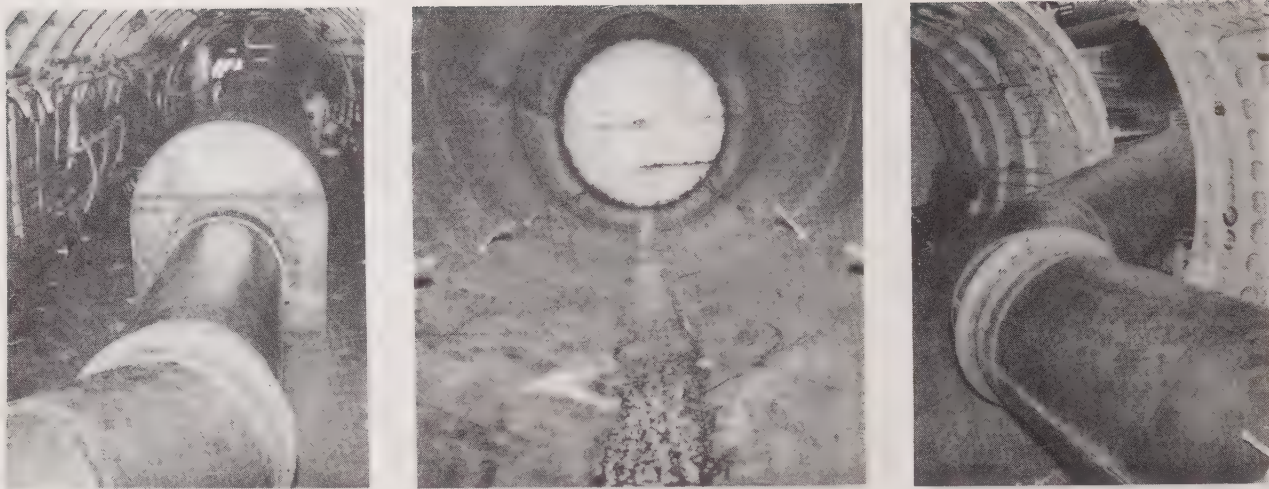


FIGURE 2.--The tank installed in the plane: (a) looking forward, (b) the interior, (c) the outlet.

As mentioned before, the primary target for this project was Spirit Lake. However, plans also called for the release of 50,000 sockeye salmon into Lost Lake. This lake, which is on the lower northwest slope of Mount Hood in the State of Oregon, is approximately one-third the size of Spirit Lake. The "over the water" time for Lost Lake was approximately 20 seconds, which called for essentially no error in flight and release technique.

The importance of good weather conditions became apparent when an initial flight was attempted on November 5, 1956. A reconnaissance flight was taken over Spirit Lake during the morning to observe "on the spot" weather conditions. Scattered clouds were in the area; however, the lake was visible, and it was very possible that these conditions would improve by the time a load of fish could arrive. In addition, Lost Lake, which was to be used as an alternate drop site, was entirely open and was expected to remain so until afternoon. However, the expected weather conditions did not occur; the Spirit Lake area was entirely covered by clouds, and the area near Lost Lake was closing in just when the plane arrived, making it necessary to return to the airport and transfer the fish to the hatchery.

On November 19, ideal weather conditions prevailed and the air lift was started

in earnest. The fish were picked up at Pangborn Airport in Wenatchee, Washington, where they had been transported by a 1-hour truck trip from the U. S. Fish and Wildlife Service's fish-cultural station at Leavenworth. Two portable fish tanks that held 1,000 gallons of water and 500 pounds of fish each were used to transport an entire load for the plane tank. A large fork lift, which would raise 7.5 tons of material approximately 20 feet in the air, was used to lift the portable tanks above the opening of the plane tank after surplus water had been removed. A 6-inch hose, with the aid of gravity flow, was used to transfer the fish from the portable tank to the plane tank (figure 4).

The loading time and flight time are shown in the table. The loading period, as shown in the table, actually includes the time spent in removing the portable tanks from the trucks and loading those tanks on the fork lift. The period of oxygen usage represents a varied amount of the loading time prior to take-off plus the actual flight time to the lakes. The relatively long period of oxygen usage during the flight on November 5 was due to the time spent on the ground after completion of the flights to the lakes and prior to reloading the fish for return to the hatchery.

On November 19-21, five flights were made to these lakes. Four drops were

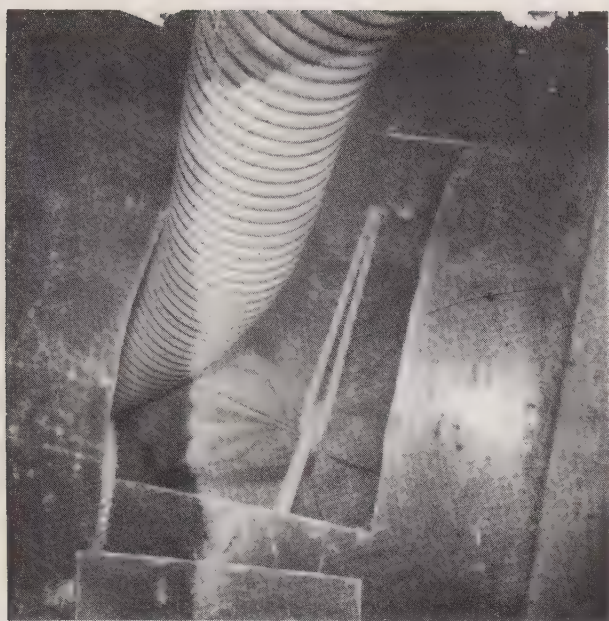


FIGURE 3.-- The release mechanism is ready for use. The overflow pipe is removed when fish are dropped.

completed at Spirit Lake and one at Lost Lake. Each plant consisted of 1,000 pounds of sockeye salmon from 1955 brood stock obtained from tributaries of Lake Wenatchee, Washington. The fish numbered 50 per pound; therefore, each plant consisted of approximately 50,000 fish, 4 to 5 inches in length.

The fish were dropped from an altitude between 150 and 300 feet above the surface of the water. Every effort was made to maintain an altitude of just under 200 feet. However, a steep approach into the lake, approximately 40 seconds over the water, and the need for a rapid change in the attitude of the airplane made this a tough but not impossible objective. When the aircraft was over the water, the manila rope which secured the release diaphragm was cut with a large knife. The actual cutting and loosening of the rope around the diaphragm required from 2 to 4 seconds. The time required for the 1,000 gallons of water and 1,000 pounds of

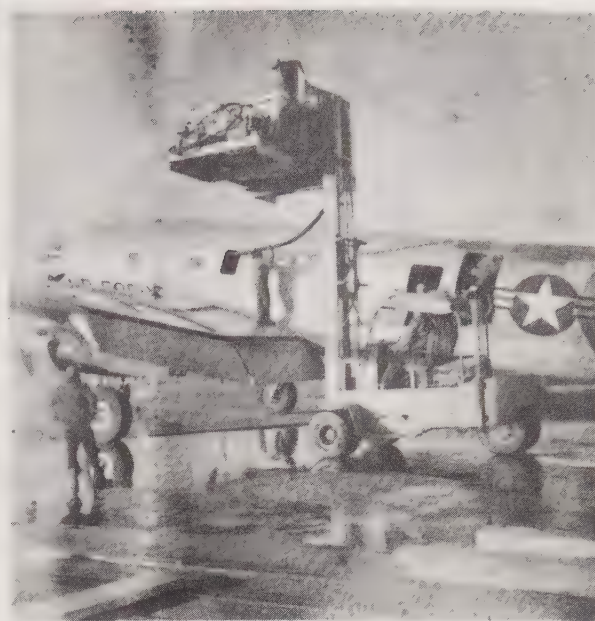


FIGURE 4.--Loading operations with 7.5-ton fork lift.

fish to be clear of the aircraft ranged from approximately 12 to 30 seconds. This time for release depended in part on how rapidly a nose-high attitude of the aircraft was assumed upon reaching a desired altitude above the water.

Aeration requirements were met by the release of oxygen into the water from the time the first fish was put into the tank until the drop into the lake was made. Oxygen cylinders containing 80 cubic feet of oxygen under pressure of 2,200 pounds per square inch were used. The amount of oxygen to be released was determined by

FLIGHTS MADE WITH FISH

<u>Area visited</u>	<u>Date</u>	<u>Loading time</u>	<u>Time oxygen used</u>	<u>Flight time</u>
(hours, minutes)				
Spirit Lake) Lost Lake) ^{1/}	Nov. 5	1:35	6:15	2:00
Spirit Lake	Nov. 19	1:30	2:10	1:00
Spirit Lake	Nov. 20	1:05	1:45	:45
Spirit Lake	Nov. 20	1:00	1:40	:45
Spirit Lake	Nov. 21	:55	1:35	:50
Lost Lake	Nov. 21	:45	1:25	1:00

^{1/} Adverse weather conditions prevented stocking this first load of fish.

reference to the bubbles rising in the water and observation of the condition of the fish. The oxygen was used at the rate of approximately 10 liters per minute.

The transfer and release technique utilized on this project appeared to be successful, as indicated by the survival at the lakes subsequent to the air drops. The fingerlings on all flights appeared to be in good condition at the time of release. Biologists were at the lakes during the drops to evaluate the release on the spot and to observe any mortality that might occur. The number of dead fish after each drop was determined as nearly as possible by observation, without actually picking up every dead fish on the lake. All drops except the second were observed. The mortality observed after the first, third, fourth, and fifth flights was determined to be approximately 1,000, 300, 200, and 3 fish, respectively, or a total of approximately 0.75 percent. Including the one unobserved air drop, it was estimated that the total mortality was less than 1 percent. The total number of fish stocked in Spirit Lake, which has a

surface area of 790 acres, was approximately 197,500. Lost Lake, with 250 surface acres, received 50,000 fish.

A close look at the progress of the air lift and the geographical area in which it was carried out reveals some of the salient features of the project. Spirit Lake is only 120 air miles or approximately 1.5 hours' round trip from the hatchery; but the same trip made by truck would have required approximately 28 hours' round trip, involving transportation of 8 loads of fish over 3 snow-covered mountain passes. A trucking trip to Lost Lake would have required efforts similar to those for Spirit Lake, with reference to time and snow-covered passes; and, in addition, the area was actually inaccessible by truck during the late fall.

A "one-trip completion time" of just under 3 hours, little handling of fish, and the indicated low mortality point toward continuation of this type of project. A second air-transport and release operation, almost identical in scope and success to the one described here, has just been completed in the late fall of 1957.



DRUGS TESTED FOR CONTROL OF KIDNEY DISEASE

At the Eastern Fish Disease Laboratory (Leetown, West Virginia) of the Bureau of Sport Fisheries and Wildlife, 34 chemotherapeutic agents have been tested for *in vitro* effectiveness on different strains of the kidney disease bacterium.

The table shows the effectiveness of various drugs tested for 21 days on brook trout infected with kidney disease by injection of the culture of the pathogen. These tests indicate the superiority of Gantrisin.

Dr. S. F. Snieszko, in charge of the laboratory,

warns, however, that subsequent use of Gantrisin gave only temporary control.

AVERAGE MORTALITIES AMONG BROOK TROUT ABOUT ONE MONTH
AFTER VARIOUS TREATMENTS FOR KIDNEY DISEASE
WERE STOPPED

Treatment	Dosage (mg./kg./day)	Percentage mortality
Erythromycin-----	40	54
Carbomycin-----	40	65.5
Tetracycline-----	40	64
Novobiocin-----	40	64
Furacin-----	100	78
Furoxone-----	100	63.5
Gantrisin-----	200	1.5
None (inoculated control lots)--	-----	62
None (uninoculated control lots)	-----	None

CAUSE OF MORTALITY OF A MIDSUMMER PLANT OF RAINBOW TROUT IN A SOUTHERN WISCONSIN LAKE, WITH NOTES ON ACCLIMATION AND LETHAL TEMPERATURES

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Madison, Wisconsin

WHEN RAINBOW TROUT were stocked on August 9, 1956, in Lower Nashotah Lake in southeastern Wisconsin, they immediately displayed distress and died soon after. This occurred when surface water temperatures were 74° F., well below the temperature reported lethal for the species (Embrey 1921, Shepard 1951). The circumstances were unusual enough, particularly in view of the fact that other lakes had been stocked at the same time with no apparent ill effects, to warrant an investigation into the causes.

Methods

In this investigation, the usual stocking procedure was followed. This meant a sample of fish from the Nevin Hatchery (Madison, Wisconsin) was placed in a tank truck filled with the same spring water supply that maintains the trout, and the trout were hauled to the stocking site at Lower Nashotah Lake in Waukesha County, Wisconsin. Lower Nashotah is a clear-water, alkaline lake with gravel, shores, and modest amounts of vegetation--no circumstances which would cause a mortality. Water temperatures of the hauling water were initially 51° and rose to 54° F. during the 1.5-hour truck haul. Surface water temperatures at the lake were 1° less than was reported at the time of the mortality (see table). At the stocking site a series of trials was set up to determine the cause of mortality.

For the first series (A) trout were transferred directly from the tank truck to the lake and released under a variety of conditions. Group 1 was held in a live box in shallow water near shore. Group 2 was released at the surface in open water, and the behavior of the fish was observed by a skin diver. Group 3 was lowered in a large cage to the upper part of the thermocline, where colder temperatures are found.

Trout for the second series (B) were given an acclimation period of 45 to 60 minutes during which the temperature of the water in the tank truck was gradually raised to that of the lake. For the third series (C) trout were given a full 24-hour acclimation period during which the hatchery water in which the trout were reared was allowed to warm up gradually to the temperature of the air. As in series A, the trout were divided into groups and given the same treatment. In the fourth

THERMOCHEMICAL DATA ON LOWER NASHOTAH LAKE, AUGUST 16, 1956

<u>Depth</u> <u>(Feet)</u>	<u>Temperature</u> <u>(° F.)</u>	<u>Dissolved</u> <u>oxygen</u>
Surface	73.00	8.4
10	70.75	8.5
20	63.00	8.2
22	60.50	4.4
30	46.00	2.2
35	41.75	----

series (D) there was also a 1-hour acclimation period for the tempered trout before stocking. A fifth series (E) was held up until the surface water temperatures of the lake had lowered to 66° F.

Test Results

The results of the tests have been summarized chronologically. Behavior of the fish, condition, and time of death were recorded. Twenty specimens were used for series A and B, group 1 and 2; and 10 specimens, for group 3 and subsequent series.

Series A, Group 1 (Live Box in Shallow Water)

Acclimation temperature: 54° F. Surface water temperature: 74° F.

10:55 a. m. --Time of stocking.

10:56 --Aimless swimming^{1/} at surface. Gasping and rapid gill movements evident.

11:00 --Slowed but continuous, aimless swimming at surface.

11:05 --4 fish balanced but motionless in lower part of box. 16 swimming aimlessly.

11:13 --First fish lost equilibrium.

11:28 --First fish ventral side up. All fish slowly, aimlessly swimming about at surface.

11:50 --First dead fish noted. 3 ventral side uppermost.

12:15 p. m. --All ventral side uppermost. 10 dead.

12:55 --All dead. 7 of 20 floating on surface.

^{1/}Aimless swimming is a fitting description of the behavior of these fish because they swam about without any direction to their movements. They bumped the sides of the live box, and they were unable to avoid the grasp of a captor.

12:55 p. m. (continued) Gill covers of dead fish closed.

Series A, Group 3 (Cage Lowered to the Thermocline)

Acclimation temperature: 54° F. Surface temperature: 74° F. Temperature at 20 feet: 63° F.

1:25 p. m. --10 fish placed in a cage and lowered to 20 feet.

1:28 --All fish swimming about freely.

1:31 --All fish oriented to the top of the cage as if seeking more shallow water.

1:34 --1 fish on side; others swimming freely.

1:48 --All fish in distress, gasping and swimming at surface of cage.

2:08 --Cage brought to surface. 1 fish dead; 2 in distress.

3:05 --3 fish in distress; other fish swimming about

3:08 --Fish released at 20 feet. No further observations. Fish had apparently dispersed in the thermocline.

Series B, Group 1 (Shallow-Water Live Box)

An effort was made to temper from the acclimation temperature of 54° F. to the lake water temperature of 73° by raising the temperature 5° every 15 minutes. No distress was observed until the water temperatures were brought up to 69° F. half an hour after the experiment began. Some of the fish began to surface, and all swam about continuously as though in distress.

Forty-five minutes after the tempering began, the experiment with group 1 was initiated. Long acclimation temperature: 54° F. Short acclimation temperature: 69° F.

12:03 p. m. --20 trout released in live box.

12:08 p.m. --16 fish surfacing.
 12:15 --All surfacing. 1 lost its equilibrium.
 12:30 --All surfacing, swimming about aimlessly. 1 in distress.
 12:46 --1 dead; 2 with ventral side uppermost; remainder in distress.
 1:15 --6 dead; 14 in distress.
 2:15 --All dead. 9 floating on surface; 11 on bottom.

Series B, Group 2
 (Open-Water Release)

This group was released at a point about 100 yards offshore in about 40 feet of water. A light onshore wind helped to recover any losses.

When released into the surface water (73° F.) at 12:59, the trout dived straight down. One specimen was observed, and that fish was seen to dive to the 15-foot level before he leveled off and began to swim horizontally. At 1:06 p.m., 2 fish had surfaced and were displaying the same behavior as the fish held in the live box. By 1:15 p.m., 10 surfaced fish were seen gasping and swimming about aimlessly. Later 3 dead were picked up on the beach.

Series B, Group 3
 (Lowered Cage)

At 2:35 p.m., this group was placed in water at a temperature of 60° F. At 3:10 they were lowered in the cage to 20 feet, where the water temperature was 63° F. At 3:24, two-thirds were in the upper part of the cage, but all appeared to be in good condition. By 3:40 there was one dead, two were in distress, and all others were in good condition. This group was then released at 20 feet, and no surfaced fish were noted after release.

On the strength of apparent success with the deep-water plants, all remaining trout (50) were placed in the holding cage and lowered to 20 feet. Upon release, they immediately popped to the surface and exhibited the same behavior as the fish held in live boxes.

Series C, Group 1'
 (Shallow-Water Live Box)

For this series 40 fish were placed in a wooden tank mounted on a truck at 9:00 a.m., and the truck was placed in the sun so that the water in the tank could gradually warm to the surface temperature of the lake. By 6:00 p.m., the temperature of the water in the tank had risen to 63° F., a warming of 10°. It remained at this temperature overnight; so the following morning sufficient warm water was added to the tank to increase the temperature to 65° F. Lake surface waters had a temperature of 73° F. A log of events was as follows:

11:22 a.m. --10 fish put in live box in shallow water.
 11:28 --All fish surfacing.
 11:57 --All fish swimming about aimlessly at the surface. Slower motions than at initiation of experiment.
 12:30 p.m. --All fish surfacing, but active.
 12:45 --5 fish were relaxed in midwater.
 1:30 --Fish alternately surfacing. Fish coordinated enough to avoid efforts to grab them.
 2:00 --All trout relaxed. No surfacing.
 2:20 --1 trout seen in distress. Trouble with balance.
 3:10 --All trout were removed to nearby warm-water hatchery (water temperature, at 71° F.) where they were kept 1 month without loss.

Series C, Group 2
 (Open-Water Release)

Ten fish were released in open water at 11:36. They dived straight down, as had the other two groups treated similarly. A single unconfirmed surfacing was observed at 11:47, but subsequently no dead fish were observed. In view of the good

survival of groups 1 and 2, the third experiment was not attempted.

Series D, Group 1
(Shallow-Water Live Box)

For this series further tempering from 65° F. to the lake temperature (73° F.) was executed before planting. Temperature increases were spread through a 1-hour period. When the water temperature reached 70° F., the fish became restless; at 70° (55 minutes after initiation of the tempering period), all were observed surfacing. The fish were committed to the shallow-water live box at 1:57 p.m. By 2:05 no further distress or surfacing was noted and all fish were acting normally.

Series E, Group 1
(Shallow-Water Live Box)

For this series, experiments were withheld until the surface water temperatures had reached 66° F. (September 10, 1956). Twenty trout were then taken from tank water at 51° F. (the acclimation temperature) and placed in the live box with water at 66°. The fish became excited and remained near the surface for 1 hour before becoming calm and relaxing near the bottom. At the end of 4 hours no mortality had been recorded and no further signs of distress observed.

Series E, Group 2
(Open-Water Release)

Twenty trout released in open water immediately disappeared. No signs of distress, surfacing, or dead fish were noted.

Discussion

These results unquestionably point to heat death (or, more popularly, "temperature shock") as the cause of this mortality. This is most evident when one observes that good survival was experienced when rainbow trout were tempered over a 24-hour period and when trout were stocked directly in cooler water at a later date.

That heat death was the cause of death is further confirmed by comparing these observations with Brett's (1944) description of behavior prior to death of bullheads. The first reaction was that of "very active swimming" and the "respiratory movements were rapid and shallow." This was followed by a "loss of equilibrium and control" and finally death. To Brett's description should be added "aimless" swimming at the surface, a behavior which appeared to be distinctly characteristic of the rainbow trout.

The work of Fry (1951) demonstrated that brook trout withstood temperature shocks as great as 36.8° F. (37.4° to 74.2° F.), and references to rainbow trout suggest that it is a much hardier species (Needham 1940) which will tolerate temperatures of 83° F. (Embrey 1921). Later work on the rainbow by Shepard (1951) under the direction of Fry, however, does point to a lethal temperature which is about the same as that for brook trout. Contrary to this laboratory evidence, in the field complete mortality of planted rainbow trout was experienced with both a higher acclimation temperature (54° F.) and a lower receiving water temperature (73° F.). As to why, there is only a series of unanswered questions. Was this a different genetic strain? Were the chemical conditions of the receiving water sufficiently different so that the heat tolerance of the trout was altered? Was there some factor in the rearing of these trout which altered their adaptability? Existence of a genetic strain of brown trout capable of withstanding warmer water has been reported in Pennsylvania.

It was found that rainbow trout could not withstand a temperature shock of 20° above the acclimation temperature of 54° F. but could tolerate a temperature shock of 15° from an acclimation temperature of 51°. Raising the acclimation temperature from 54° through a 24-hour period to 65° F. enabled the trout to withstand water at a temperature of 74° F. with only minor distress for a short period. In all cases it was noted that 70° was approximately the threshold above which distress occurred.

Knowledge of the consequences of temperature shock to rainbow and brown trout is not entirely new to Wisconsin workers. In an unpublished memorandum with incomplete data, Woodbury and Roecker stated that a 10° rising shock or 1- to 3-hour tempering did not affect brook trout but was fatal in most cases to brown trout.^{2/} Rainbows reportedly took the 10° shock better than brown but not so well as brook trout.

Until recent years Wisconsin avoided stocking trout in waters at temperatures over 70°. However, this rule was somewhat relaxed in the light of experimental evidence originating elsewhere. The recent experience with stocking trout in lakes during midsummer when surface water temperatures are above 70° has been varied. Trout stocked in small lakes gave little or no sign of distress, whereas trout stocked in large lakes (more than 50 acres) experienced mortalities. The reasons can be pieced together from these data. Trout lowered in a cage to the cool water of the thermocline (20 feet) rose to the top of the cage as if seeking shallower water. Those lowered and released in cool water 20 feet deep immediately came to the surface, whereas those released at the surface in warm water dived straight down. Apparently the trout becomes a victim of conflicting drives, in an effort to adjust to his environment.^{3/} He will dive to depths of about 15 feet in his initial search for cool water before it hurts him and the need for pressure acclimation becomes overwhelming; thus, if the thermocline lies within the range of this initial dive, the planted trout will be quite safe in

^{2/}Woodbury, L. A., and A. W. Roecker. First interim report of the effect of thermal shock on various ages and species of trout. (Mimeo.) Wis. Cons. Dept. July 7, 1940.

^{3/}Theoretically the trout, a physostome, with an open swim bladder should be able to adjust to any depth (Jones 1952). However, this did not occur without hurt. It is conceivable that trout reared in the shallow water of raceways acquire this tropism and do not adapt to depths.

the cool water. As small lakes ordinarily have thermoclines within this range, stocking will be successful regardless of temperature shock. It was found, however, that the trout could become acclimated in time to the sudden increases in depth (20 feet) but not to sudden sharp temperature changes.

Management Recommendations

Certain recommendations of significance to management are warranted. Rainbow trout should not be stocked when the temperature of the receiving water is 20° more than the holding water (usually spring water of about 50° F.). Before they are stocked in water that is above 70° F., rainbow trout should be acclimated through a 24-hour period to a temperature within 10° F. of the receiving water.

These experiments establish heat as the cause of death for trout planted in Lower Nashotah Lake. Heat death was avoided in small lakes because the thermocline with its cool water was more accessible than it was in large lakes. Thus, stocking should be withheld when the thermocline is not within 15 feet of the surface. Restrictions on the size of gear and the duration of the acclimatization period would rule out mechanical attempts to stock trout within the thermocline in lakes having thermoclines below 15 feet.

Acknowledgments

The advice and manuscript review of Dr. A. E. Hasler and Dr. Edward Schnerberger are gratefully acknowledged by the writer.

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SMALL-MESH GILL NETS USED IN WYOMING STUDIES

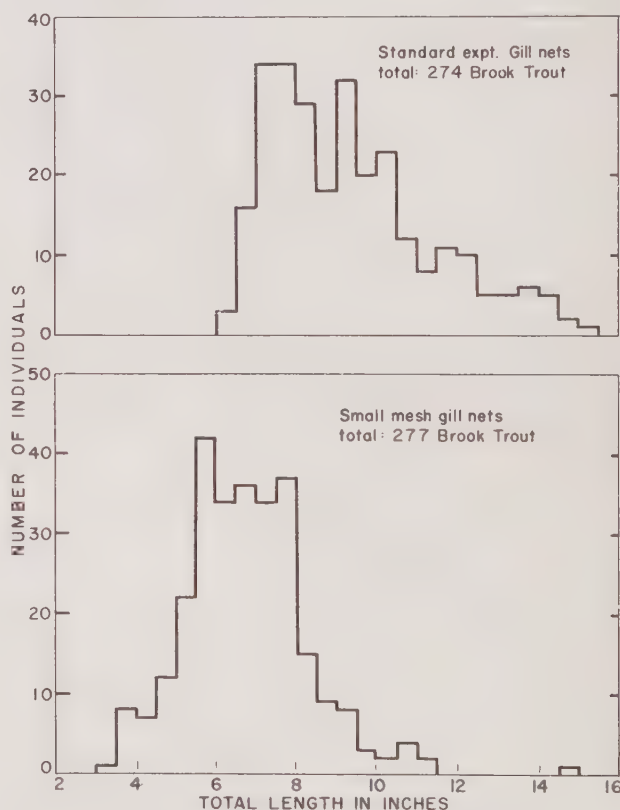
Trials during 1956 demonstrated the effectiveness of the small-mesh gill net as a fishery-management tool, according to George D. Holton, fishery biologist of the Wyoming Game and Fish Commission.

For a survey of mountain lakes in southeastern Wyoming, fishery management crews used nylon gill nets of two types: first, the standard experimental gill net, 200 feet long, 6 feet deep, having eight 25-foot sections made of 3/4-, 1-, 1-1/4-, 1-1/2-, 1-3/4-, 2-, 2-1/4-, and 2-1/2-inch square mesh, respectively; and second, the net of smaller mesh obtained from Japan, measuring 100 feet long, 6 feet deep, and having four 25-foot sections made of 3/8-, 1/2-, 5/8-, and 3/4-inch square mesh, respectively. Each net was set overnight with its smallest mesh toward the shore.

The length frequency of the brook trout taken in each type of net during the survey is shown in the accompanying graph. These data are from 13 lakes in which both the standard experimental nets (19 sets) and the small-mesh nets (16 sets) captured brook trout.

The small-mesh nets sampled brook trout that were only 3-4/10 inches long. These nets proved valuable in assessing the reproduction of trout in the lakes that were being studied.

"In another study," Mr. Holton reports, "the small-mesh nets are used to make periodic checks on the growth of fingerling hatchery trout planted in lakes."



(Drawing courtesy of Mr. Holton)

CONSTRUCTION OF A TWO-WAY WEIR FOR THE ENUMERATION OF SALMON MIGRANTS

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ONE OF THE BASIC NEEDS OF MANY FISHERY RESEARCH PROBLEMS is an accurate enumeration of the fish migrations. Although many weirs have been designed to meet this need, few of them may be operated effectively and economically to count both upstream- and downstream-migrating salmon. The weir described here successfully provided such a two-way count of adult and fingerling blueback salmon (*Oncorhynchus nerka*) near Redfish Lake, Idaho.

Description of Site

Redfish Lake Creek extends 1-1/2 miles from Big Redfish Lake to Little Redfish Lake and 3/4 mile from Little Redfish to the confluence with the Salmon River about 5 miles above Stanley, Idaho, or 377 miles above the mouth of the Snake River. The flow of Redfish Lake Creek varies from 50 cubic feet per second to an estimated 275 cubic feet per second. The stream is 56 feet wide at the site of the weir.

Design and Construction

A permanent structure of unique and simple construction was designed so that it could be modified easily either to count upstream migrants or to count the downstream migrants and determine the distribution across the stream. The weir was built in April and May 1955 during extreme low water.

A unique feature of this structure is the foundation of steel H-beams driven

into the stream bed with a jackhammer or concrete breaker. An adapter fits over the top of the sharpened steel beams to engage the jackhammer. Installation is rapid, and the resulting weir is durable.

The H-beams of the Redfish Creek weir were driven at 6-foot intervals in 2 rows 9 feet 8 inches apart. The beams were 6 feet 8 inches long and were driven 4 to 5 feet into the bed of the stream and then cut off at an even 4 inches above the water surface with an acetylene torch.

Steel brackets (3-1/2" x 6" x 5/16") were welded at the water surface to the upstream face of the upstream row of H-beams. The 4" x 12" sill was bolted to these brackets as shown in figure 1.

Modified A-frames of 4" x 6" timbers were next constructed with the legs of the frame 6 feet long and forming a 45° angle to the horizontal. Prefabricated steel brackets were bolted to the A-frames and then to the H-beams. The A-frames were trued and tied together when the 2" x 12" planks of the upper walk were fastened into place with lag screws.

Deadmen constructed of 4-inch H-beam material welded together at right angles were buried in the banks and bolted to the end A-frames. The end A-frames were sheathed on the outside with 3" x 6" timbers, and the abutments were built of rock and earth.

Next the 3" x 6" stoplogs were placed in the channels of the upstream row of H-beams and buried deep enough to prevent a "blowout." Chicken wire was placed in front of the weir as a further precaution to

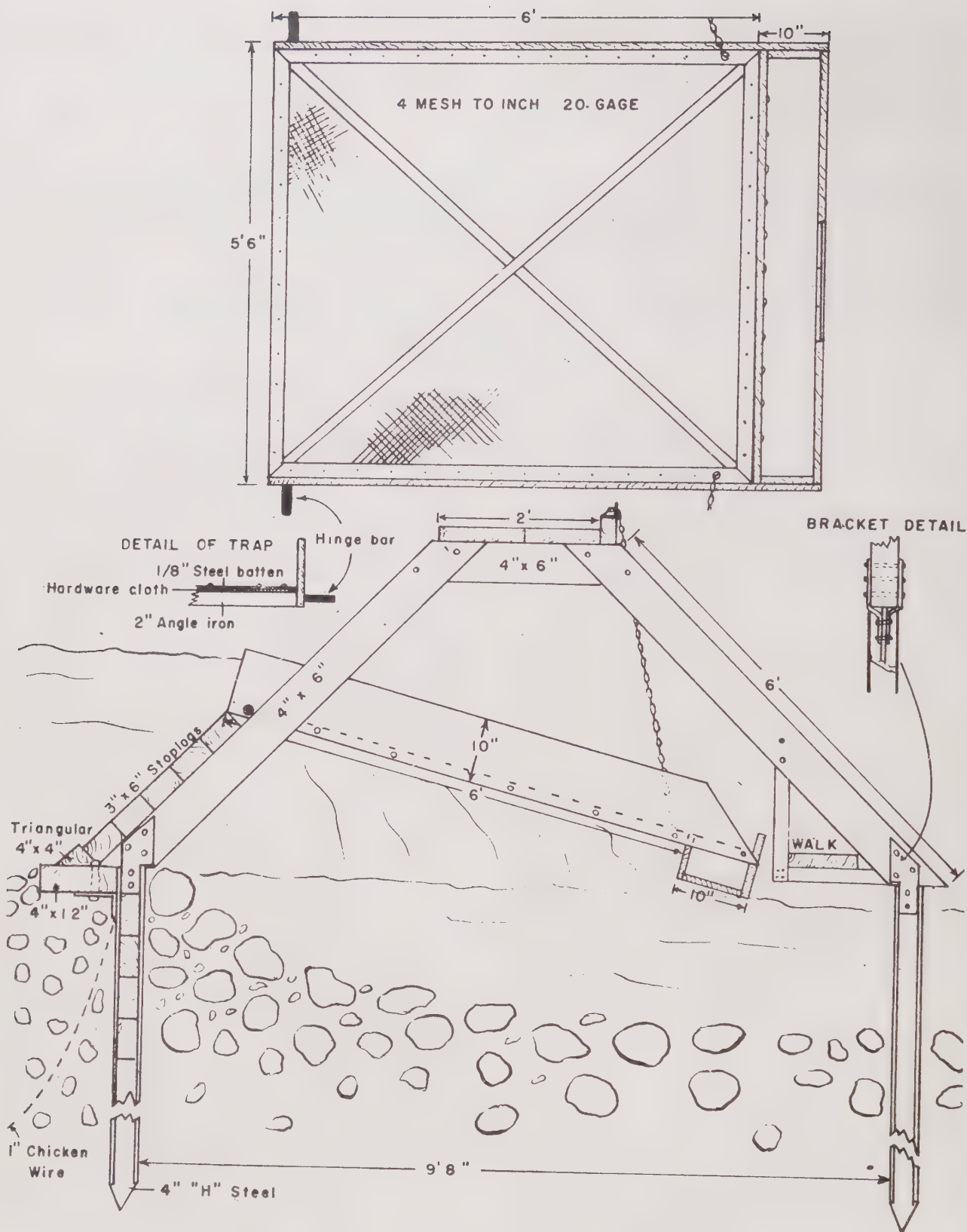


FIGURE 1.--Basic structure and downstream trap.



FIGURE 2.--Basic structure of Redfish Lake Creek weir.

stabilize the bottom and prevent undercutting. The front of the weir was back-filled as shown in the figures, and heavy rock was placed behind the row of stoplogs as a final precaution to prevent undercutting by the spill. Thus, a small dam was created with a head of about 1 foot (figure 2).

Downstream Weir

The weir was prepared for downstream trapping by fastening the triangular piece (4" x 4") on the sill as shown in figure 1. This piece, which served as a footing for the stoplogs, was secured permanently except in the middle section, where temporary fastenings permitted removal for installation of the upstream trap. Stoplog guides (1" x 3" x 3') were fastened vertically with spikes to the longitudinal center of the upstream legs of the A-frame. The 3" x 6" stoplogs were then cut and put in place.

The traps were constructed of angle iron (2" x 2" x 1/8") welded to the proper dimensions. The diagonal braces were bars (1-1/4" x 1/8"), and the 20-gauge galvanized hardware cloth had 4 meshes to the inch. The hardware cloth was bolted to the frame with an iron-bar batten strip (1" x 1/8") and 1/4-inch stove bolts. The trough and side boards were constructed of 1-inch lumber bolted to the trap frame as shown in figure 1.



FIGURE 3.--Downstream weir, Redfish Lake Creek.

A screened opening was necessary in the upper part of the trough to prevent overflow and loss of fish. The trap was hinged on the A-frame by means of a 3/4-inch bar extending through the trap or by means of long 3/4-inch bolts threaded all the way and using 2 nuts. The downstream end of the trap was supported by the chain bridle fastened to the upper walkway.

The proper flow and angle of the trap were achieved by adjusting the chain and the number of stoplogs employed. The flow of water reached almost to the trough when the trap was fishing properly. In this way the water carried the fish to the lip of the trough.

The downstream weir on Redfish Lake Creek is shown in figure 3.

Upstream Weir

The only alterations necessary to the basic structure when installing the upstream weir were the removal of the triangular sill piece in section 5 and the addition of the 4" x 4" stringer on the upstream face of the weir.

The picket sections were 5 feet wide and made of 1" x 3" lumber. The pickets were 5 feet long with the ends cut parallel at a 45° angle. They were spaced 1-1/4 inches apart and secured with a header on the bottom and a strip in the middle and near the top. The sections were placed on the sill in front of the triangular piece and

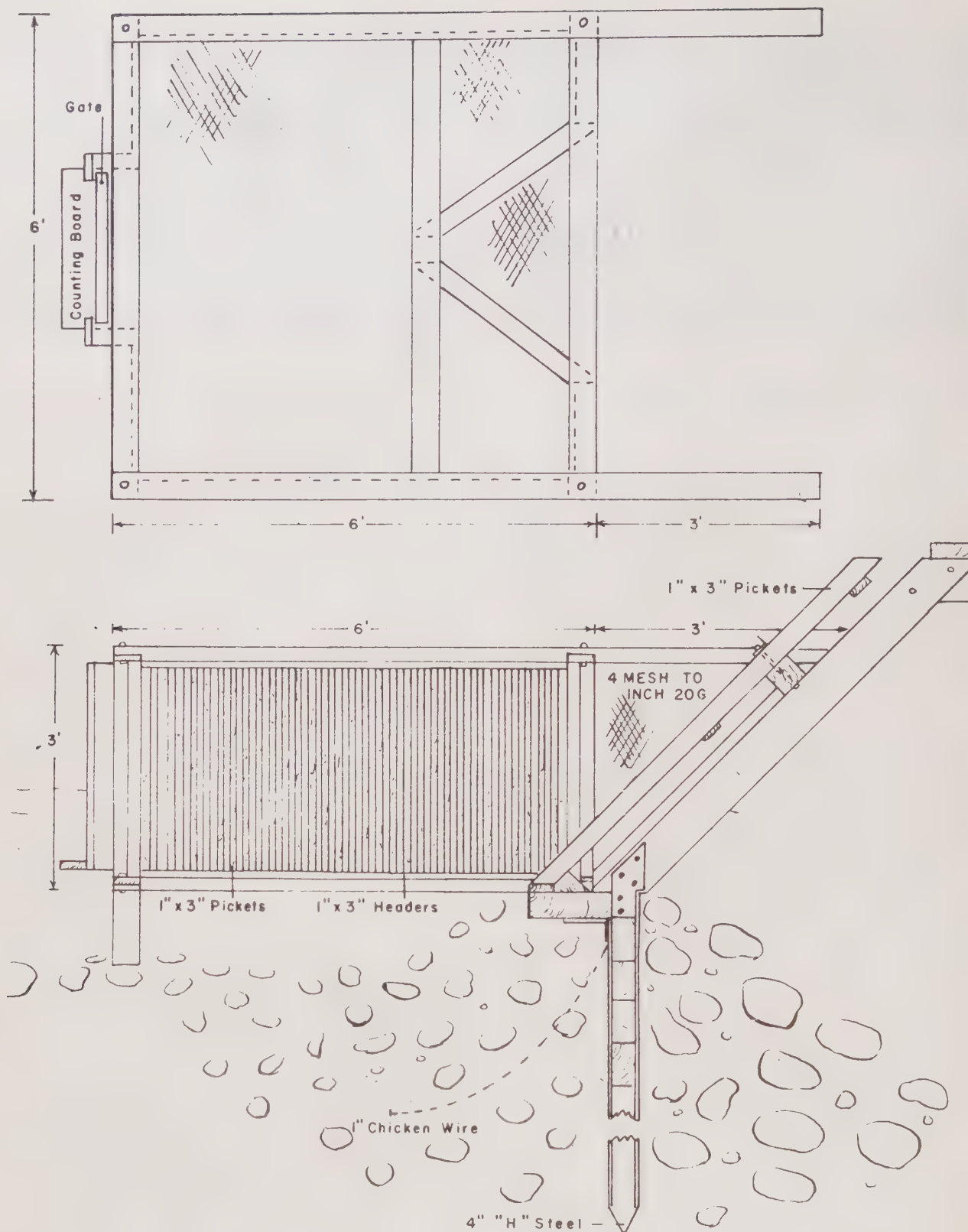


FIGURE 4.--Basic structure and upstream trap.

fastened to the 4" x 4" stringer with a cleat and bolt.

The upstream trap was built with a 2" x 4" framework (6' x 6' x 3'). The trap included a conventional tunnel constructed of pickets narrowing to a 4-inch entrance, an upstream gate, and a counting board (figure 4). The pickets of the trap were spaced 1 inch apart and were built in separate sections with headers on both ends. The bottom, hinged top, and triangular leads were constructed of hardware cloth. The upstream trap was placed and secured to the weir as shown in figure 4. Additional weights consisting of H-beams were placed upstream and downstream on the trap to eliminate the possibility of movement.

Discussion

This weir has been in operation for two seasons and has proved satisfactory in most respects. No difficulties, mortalities, or delays were noted in passing the adults upstream. Mortalities occurring during the downstream trapping were insignificant.

The weir has several advantages, a few of which are as follows:

1. Rapid construction. The structure was completed in 10 days by 4 men working under adverse conditions of cold and snow.

2. Economy. The cost of materials and labor was about \$3,000.

3. Ease of operation. The upstream or the downstream modifications can be made by 2 men in less than 2 days. Under ordinary circumstances, 1 man can operate the weir with little difficulty.

The disadvantages of the weir are as follows:

1. It is not adaptable to all types of bottom, as the steel may be difficult to drive in heavy rock or may be unstable in loose sand.

2. Its construction requires considerable equipment--a compressor and jackhammer, acetylene equipment, welder, and bulldozer.

3. The downstream traps are not completely adjustable and may be flooded out in very high water.

Acknowledgments

Mr. Leonard Fulton was in charge of the project and thus was instrumental in initiating and arranging for the construction of the weir. Mr. R. J. Holcomb provided invaluable advice and assistance in the design and in the actual construction of the weir. Mr. F. R. Hauck of the Idaho Fish and Game Department was very cooperative and arranged financial aid from his department.



In general, egg size among North American salmonidae is correlated with average size of species. The four species of fluvial anadromous Oncorhynchus have larger eggs than the sockeye, O. nerka, or species of the other genera.

Because of lack of knowledge of their identities, few trout populations in the United States are well suited for studies on races. The Yellowstone cutthroat trout, Salmo clarki lewisi (Girard), however, offers an admirable opportunity for such studies. This stock of trout is supported within a closed system consisting of Yellowstone Lake and the Upper Yellowstone River, and possibly part of the Snake River drainage; and no introduction of another species of trout has been made within the history of fishery work.

ESCAPE OF FISH OVER SPILLWAYS

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THERE IS SOME CONTROVERSY as to whether there is a significant loss of fish over the spillways of lakes. There is also some question as to what sizes and species are involved. The present study was designed to obtain more information on this matter. Essentially the study consisted of trapping in wire nets fish escaping over the spillways of two artificial lakes.

The Lakes

Little Grassy Lake is an impoundment approximately 10 miles southeast of Carbondale, in southern Illinois. At spillway elevation, the lake covers an area of approximately 1,200 surface acres and has a maximum depth of 60 feet.

Lake Murphysboro is an impoundment approximately 2 miles northwest of Murphysboro, in southern Illinois. At spillway elevation, it covers an area of approximately 160 surface acres and has a maximum depth of 30 feet.

Both lakes receive their water from surface runoff and are equipped with concrete spillways. The lakes overflow only during rainy seasons.

Fish Populations

It is important to consider what fish species are present in the lakes and their abundance in order to determine the correlation between species taken in the trap and those occurring in the lake. During a previous investigation of Little Grassy Lake, 14 species were collected.^{1/} These were taken by electrical shocker, in wire

baskets and gill nets. In the probable order of abundance in the lake, the 14 species were as follows: gizzard shad (Dorosoma cepedianum), carp (Cyprinus carpio), longear sunfish (Lepomis megalotis), bluegill (Lepomis macrochirus), white crappie (Pomoxis annularis), largemouth bass (Micropterus salmoides), black crappie (Pomoxis nigro-maculatus), green sunfish (Lepomis cyanellus), spotted sucker (Minytrema melanops), yellow bullhead (Ictalurus natalis), black bullhead (Ictalurus melas), warmouth (Chaenobryttus gulosus), golden shiner (Notemigonus crysoleucas), and the mud pickerel (Esox americanus).

During previous investigations of Lake Murphysboro, 12 species were collected. These were taken by electrical shocker and wire baskets. In the probable order of abundance in the lake, the species were as follows: bluegill, largemouth bass, red-ear sunfish (Lepomis microlophus), white crappie, warmouth, green sunfish, yellow bullhead, channel catfish (Ictalurus lacustris), western lake chubsucker (Erimyzon sucetta), stoneroller (Campostoma anomalum), bluntnose minnow (Pimephales notatus), and golden shiner.

Procedure

The nets used to capture fish escaping over the spillways were constructed of

^{1/}Walker, G. W. A fish population study of an artificial lake in southern Illinois. Southern Illinois University, master's thesis. 33 pp. 1951.



Trap in place on Lake Murphysboro spillway.

(Photograph courtesy of the author)

1-inch wire netting for the Little Grassy Lake spillway and of 0.25-inch hardware cloth for the Lake Murphysboro spillway. In both instances the netting was secured at the bottom by means of nails driven into the expansion joints of the spillway. The top of the wire was supported upward and against the current by guy wires. The resulting structures were in the form of seine-line traps (see figure). A portion of the water coming over the spillways passed through the traps. The Little Grassy Lake net was 40 feet long and strained 41 percent of the water flowing over the spillway. The Lake Murphysboro net was 24 feet long and strained 33 percent of the water. To convert the catches to total losses for Little Grassy Lake and Lake Murphysboro, the losses

observed were multiplied by 2.5 and 3.0, respectively.

Once put in place, the nets were permitted to remain there until the study was completed. When there was a rain, the nets were cleaned. When overflow commenced, observations were started. Observations included depth of water over spillway, period of overflow, and numbers of each species, and size of each fish taken.

During the 5-month period of observation on Little Grassy Lake, a total of 665 fish came over the spillway, involving a total of 885 hours of overflow (table 1). Adult gizzard shad made up 46 percent of the actual catch. Adult carp, largemouth bass, spotted sucker, and bluegill were about equally represented in the catch: they made up 14, 11, 9, and 8 percent, respectively. The largest loss of fish occurred in April 1955.

During the 23-month period of observation on Lake Murphysboro, a total of 10,182 fish came over the spillway, involving a total of 1,737 hours of overflow (table 2). The major part of the loss was composed of largemouth bass, 6 to 12 inches in length: these formed 53 percent of the total catch. Adult redear, young bluegill, and young green sunfish made up 15, 11, and 7 percent, respectively, of the total catch. The largest losses occurred in June 1954 and April 1955.

TABLE 1.--Fish escapement over 41 percent of the spillway on Little Grassy Lake

Month and year	Extremes of water depth over spillway (inches)	Number of hours of overflow	Actual number of fish caught									
			Gizzard shad		Carp	Largemouth bass		Bluegill		Spotted sucker	Misc.	
			(Length classes in inches)									(1/)
			1-7	8-13	13-26	1-9	10-14	1-5	6-8	11-14		
Dec., 1954	2	47	0	0	0	0	0	0	0	0	0	
Jan., 1955	1	89	0	0	0	0	0	0	0	0	0	
Feb., 1955	4	72	1	3	0	1	0	0	4	9	7	
Mar., 1955	21	504	9	9	0	0	1	1	8	13	9	
Apr., 1955	5	173	7	93	36	0	29	0	11	2	13	
Total-----		885	17	105	36	1	30	1	23	24	29	

1/The miscellaneous fishes were longear sunfish, green sunfish, yellow bullhead, black bullhead, mud pickerel, and golden shiner, in order of abundance.

TABLE 2.--Fish escapement over 33 percent of the spillway on Lake Murphysboro

Month and year	Extremes of water depth over spillway (inches)	Number of hours of overflow	Actual number of fish caught								Misc (1/)
			Bass		Redear sunfish (Length classes in inches)		Bluegill		Green sunfish		
			1-5	6-12	1-5	6-10	1-5	6-9	1-5	6-9	
May, 1954	2	72	1	0	0	0	2	0	0	0	0
June, 1954	2	95	182	280	50	21	27	14	66	0	4
Dec., 1954	1	45	0	0	0	0	0	0	1	0	0
Jan., 1955	1	95	0	0	2	0	0	0	13	0	0
Feb., 1955	1	74	2	0	0	0	0	0	9	4	0
Mar., 1955	3	441	2	359	4	4	16	1	67	1	8
Apr., 1955	1	222	0	1,045	41	485	9	43	35	3	3
May, 1955	1	209	1	1	0	0	105	0	14	0	4
June, 1955	4	66	31	1	0	0	214	2	0	0	0
Feb., 1956	3	231	1	0	0	0	0	0	3	0	5
Mar., 1956	2	100	1	125	1	1	1	9	16	1	11
Apr., 1956	.25	87	0	0	1	0	1	0	4	2	14
Total-----		1,737	221	1,811	99	511	375	69	248	11	49

1/The miscellaneous fishes were golden shiner, stoneroller, yellow bullhead, lake chub sucker, and bluntnose, in order of abundance.

Discussion

Whether or not the escape of fish over the spillway is important in management might best be established by considering the following points:

1. Are all species of fish affected? In Lake Murphysboro, where the data are most conclusive, five species were dominant. Four of these were well represented in the escapement; however, the fifth dominant species, white crappie, did not occur in the catch. For a large Ohio impoundment, Clark (1942) found that loss figures for crappie were particularly high.

2. Are fish lost in any significant number? In Lake Murphysboro, during a 23-month period of observation, approximately 10,000 fish came over the spillway. The majority of these were adults. In a 160-acre lake such a loss of fish must be considered as significant, even though it may be argued that the loss is beneficial.

3. What sizes of fish are lost over spillways? On Little Grassy Lake the mesh size of the net did not permit the catching of small fish. The net used on Lake Murphysboro was constructed of 1/4-

inch mesh. Adults were predominant in the catch at Lake Murphysboro. For example, of 2,032 largemouth bass taken, 1,811 were 6 to 12 inches in length.

4. Under what conditions do losses occur? One would assume that there would be a direct relationship between depth of water flowing over the spillway and loss of fish, but such is not the case. It appears that season is more important (table 2). The heavy losses appear to occur in the spring. The erratic nature of the loss probably accounts for the controversy that has existed with regard to fish losses over spillways.

Management Significance

The conclusion drawn from the study is that fish are lost in great numbers over spillways during the period of flooding. All sizes and apparently most species are involved. It therefore seems advisable to install a wire screen, in a foot or more of water, in front of spillways to prevent such a loss of game fish. In some cases, game fish going over the spillway might enrich the populations of the streams below the lakes.

A trap on the spillway is an ideal place to obtain brood-size fish for stocking purposes. Many of the fish taken in the present study were used in this manner.

Summary

Among fishery biologists there is controversy as to whether there is a significant loss of fish over the spillways of lakes and ponds. To investigate this matter, wire netting was rigged on the spillways of two lakes in such a manner as to strain the water passing over the spillways. The data indicated that the loss of larger fish in the spring was considerable.

The loss from a 160-acre lake during the first year of sampling included 546 largemouth bass 1 to 5 inches long and 840 largemouth bass 6 to 12 inches long. A total of 342 redear and bluegill (a third of which were of catchable size) were taken. The loss during the second year of sampling included 108 largemouth bass 1 to 5 inches long and 4,218 largemouth bass 6 to 12 inches long. A total of 42 redear

and bluegill (half of which were of catchable size) were also taken.

Heavy loss of fish appeared to be correlated with season rather than with magnitude of overflow. The greatest losses occurred in June 1954 and April 1955. It is also of interest that heavy losses occurred in short periods of time.

Acknowledgment

This work was done under the direction of Dr. W. M. Lewis. Mr. Sam Parr made this study possible by placing transportation and other facilities at my disposal. I gratefully acknowledge also the help of my wife and the staff of the Cooperative Fisheries Laboratory.

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THE PHYSIOLOGY OF THE PITUITARY GLAND OF FISHES, by Grace E. Pickford and James W. Atz. 637 pages. New York Zoological Society. \$6 postpaid from the Publications Office, New York Zoological Park, New York 60, New York. 1957.

This publication is a review of the extensive and widely dispersed literature on the physiology of the pituitary gland of fishes. The nature of the fish pituitary gland hormones and their sites of origin are discussed. The regulation of the target organs and their functions is reviewed in relation to the biology of the organism as a whole, and a comparison is made with the knowledge of pituitary physiology in higher vertebrates.

Methods of treating fishes with gonadotropic hormones in order to induce reproduction in captivity are described in detail, including the extensive fish-cultural work that has been performed in Brazil, Japan,

and the U. S. S. R. All known experiments, up to June 1956, concerning the pituitary gland and pituitary physiology of fishes are summarized in 54 tables. An addendum covers, in so far as possible, the literature for the last months of 1956. The technique of hypophysectomy is discussed in an appendix.

There is a combined bibliography and author index, consisting of 1,905 complete bibliographic references, accompanied by page references to citations in the text and tables. Cross references to co-authors are included. There is a detailed subject index.

QUINALDINE, A NEW ANESTHETIC FOR FISH

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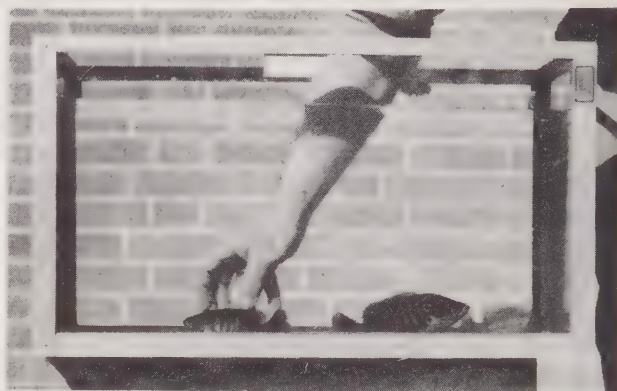
ANESTHETICS are widely used in transporting and handling fish. Some of the chemicals already in use for this purpose include: sodium amytal, thiopental sodium, urethane, tricaine methanesulfonate (M.S. 222), chlorobutanol, ether, and carbon dioxide. The present study was designed to find a safe, more effective, and economical drug--one that might make possible anesthetization of fish populations in their natural habitat. As a result of screening a great many compounds, it was found that quinaldine, a chemical heretofore unrecognized as a fish anesthetic, exhibits the desired characteristics. This drug is of sufficient effectiveness to be considered as a chemical for large-scale anesthetization.

Quinaldine (2-methylquinoline) is found in coal tar. This chemical apparently is not used in medicine, though other quinolines have some antiseptic value and have been used as antimalarials and pesticides. Quinaldine and other quinolines are used in the manufacture of dyes and explosives.

Effect on Fish

The nature of the effect of quinaldine on fish is not known, but fish exposed to this chemical exhibit a state of quiescence similar to the effects caused by the

In part from a master's thesis presented to the faculty, Department of Zoology, Southern Illinois University. This study was sponsored jointly by the Illinois Department of Conservation and the Co-operative Fisheries Research Laboratory of Southern Illinois University.



Fish anesthetized by quinaldine do not respond to gentle handling.

(Courtesy of the author)

barbiturates which act as depressants of the central nervous system. When live fish in water are treated with effective concentrations of quinaldine, a cessation of movement occurs in 45 seconds to 6 minutes. Apparent movement after drug-ging generally is confined to those motions required for breathing. The drugged fish rest in a position which varies somewhat with the species of fish and with the concentration of the chemical used. While in the anesthetized state, the muscles of the fish are relaxed. The drugged fish do not respond to gentle handling. They can be moved about in the water by hand without offering any resistance (see figure). If handled roughly, if grasped by the tail, or if the side of their container is struck sharply, the fish may react by swimming a short distance and coming to rest again.

When anesthetized fish are transferred from a solution of quinaldine to fresh

EFFECTS OF DIFFERENT CONCENTRATIONS OF QUINALDINE UPON FISH

[Water temperature of experiments, 74° - 79° F. unless otherwise indicated]

Species	Number used	Quinaldine (p.p.m.)	Time to anesthetize (minutes)	Time held in solution (hours)	Time of recovery when moved to fresh water (minutes)
Green sunfish----	2	2.5	(1/)	-----	-----
Do.-----	3	5.0	1.0	48.0	10.0
Do.-----	5	2/7.0	6.0	5.0	(3/)
Do.-----	2	4/7.0	2.0	27.0	10.0
Do.-----	2	10.0	.7	18.0	4.0
Do.-----	2	20.0	.5	11.0	5.0
White crappie----	2	5/7.5	2.0	23.0	5.0
Yellow bullhead---	2	6.0	3.0	2.0	(6/)
Do.-----	1	4/7.0	2.0	70.0	5.0
Golden shiner-----	2	7.0	(1/)	-----	-----
Do.-----	2	8.2	(1/)	-----	-----
Do.-----	2	12.0	2.0	4.5	.7
Goldfish-----	2	5/7.0	4.0	27.0	10.0
Do.-----	1	12.0	1.0	22.0	5.0

^{1/} The extent of anesthesia was not sufficient to qualify.^{2/} Experiment conducted in 780-liter tank.^{3/} After 5 hours of exposure, the fish began to recover in the test solution.^{4/} Experiments conducted at water temperature of 50° F.^{5/} Experiments conducted at water temperature of 54° F.^{6/} After 2 hours of exposure, the fish began to recover in the test solution.

water, recovery commences at once; within a few minutes they are reacting in a normal manner to various stimuli. Time for total recovery ranges from 1 to 10 minutes. Seemingly, recovery time is proportional to the length of time that the fish are exposed to quinaldine and is also influenced by the relative strength of the concentration.

The concentration necessary to achieve an anesthetic effect on fish is low (see table). With the species tested, from 5 to 12 p.p.m. quinaldine was sufficient to produce the desired state of anesthesia.

Fish can be exposed to effective concentrations of quinaldine for relatively long periods without harmful effect. Some species were left in test solutions for as long as 2 and 3 days, but they recovered in a matter of a few minutes when transferred to fresh water. These fish were observed for several weeks after such exposures in order to be certain that there were no observable ill effects as a result of the tests.

A solution of quinaldine will maintain its anesthetic qualities for several days under laboratory conditions, the time depending on the strength of the concentration. A concentration of 10 p.p.m. maintained its effectiveness for 6 days in a laboratory aquarium; under field conditions, however, it is doubtful that this long a period of effectiveness would be sustained.

Although quinaldine at low concentrations has a marked effect on fish, the fish can tolerate considerably higher concentrations without exhibiting acute toxic reactions. Green sunfish were held in a concentration of 20 p.p.m. quinaldine for 11 hours without ill effects. This is 3 times the concentration necessary to anesthetize this species.

The toxicity of quinaldine to animals other than fish has been studied. For rats the LD₅₀ by oral dose is 1,230 milligrams per kilogram of body weight, and for rabbits a cutaneous injection of 1,870 milligrams per kilogram of body weight is

required for LD₅₀ (Spector 1956). This indicates a relatively low toxicity of quinaldine to these animals. No cancer tests of quinaldine are known.

Properties of Quinaldine

Quinaldine has a density of 1.059 and is slightly soluble in water. In the laboratory experiments, quinaldine was generally used in an acetone solution of no more than 1:10,000 in final concentration. Acetone by itself has no effect on fish at this or even somewhat higher concentrations. Quinaldine used without a solvent is just as effective, but a better and quicker

distribution is achieved by diluting it with acetone.

The effectiveness of quinaldine at low water temperatures of 50° to 54° F. is about the same as at higher temperatures of 74° to 79° F. There are some indications that a longer time may be required for anesthetized fish to recover at the lower temperatures.

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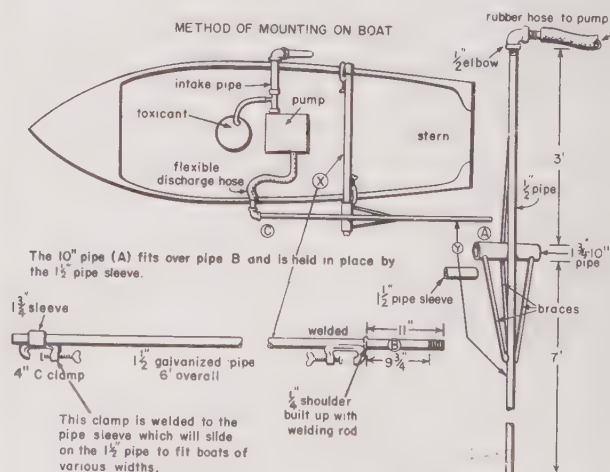
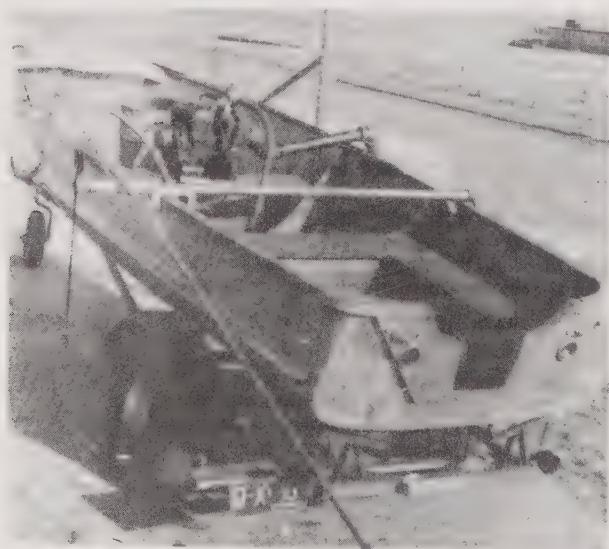


APPARATUS FOR DISCHARGING LIQUID FISH TOXICANT UNDER WATER

"For even dispersion of a liquid fish toxicant," writes F. T. Williams, fishery biologist of the Wyoming Game and Fish Commission, "an apparatus was developed to force the toxicant into the water, well below the surface."

To control the depth of discharge, the operator raises or lowers the underwater unit pivoting on the 1.5-inch pipe attached to the gunwales. The hose from the pump must be long and flexible enough for free movement of the discharge pipe. Move-

ment of the underwater unit is controlled from point C. As much as 16 feet of 0.5-inch pipe, below the pivot point, can be raised from the water without danger of breaking; a longer pipe should not be lifted wholly from the water without support. The discharge end of the pipe should point to the stern; if the pipe strikes an object under water, it will be deflected upward, without damage. The intake from water to pump should be of pipe, not hose, as the pipe is easier to attach and keep in place.



(Illustrations courtesy of Mr. Williams)

THE EFFECTS OF ACID MINE POLLUTION ON THE FISH POPULATION OF GOOSE CREEK, CLAY COUNTY, KENTUCKY

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GOOSE CREEK, a tributary of the South Fork of the Kentucky River, was polluted on April 2, 1957, by acid waters from an operating coal mine. The mine responsible for the pollution is located about 4 miles from the mouth of Island Creek, which empties into Goose Creek 2 miles below the town of Manchester.

Sixteen and three-tenths miles of the stream was reportedly polluted, effecting a fish kill over the same area.

On April 22 and 23 an electric shocker was employed on Goose Creek in order to determine the extent of the reduction of the fish population. During the 3-week period between the time that the pollution occurred and the time that the study was begun, the water level had risen and subsided once.

The standing fish population was measured by shocking two similar areas, one above and one below the source of pollution, and comparing them.

A total area of 2.07 acres above the mouth of Island Creek was sampled. This area produced 126.9 pounds of fish, or 61.3 pounds per acre (table 1).

Below the mouth of Island Creek, a total area of 3.44 acres was sampled. The total weight of fish recovered in this area was

18.5 pounds, or 5.38 pounds per acre (table 2).

It was estimated by the crew of 6 men conducting the study that 70 percent of the shocked fish were recovered.

The total poundage of fish in the area above Island Creek was nearly 12 times as great as the total poundage of fish in the polluted area.

In order to estimate the extent of damage to the game- and pan-fish population, an actual creel value was placed on each fish of catchable size. The sum of \$1.22 per fish was calculated from creel-census

TABLE 1.--Fish population of Goose Creek in an unpolluted area above Island Creek on April 22, 1957

Species	Number	Length (inches)	Weight (pounds)
Game Fish-----	17	-----	15.0
Largemouth bass--	3	10.6 - 13.8	3.1
Kentucky bass----	1	8.6	.3
Smallmouth bass--	13	5.6 - 16.6	11.6
Pan Fish-----	72	-----	21.8
Rock bass-----	48	4.2 - 10.8	20.3
Longear sunfish--	24	3.4 - 4.9	1.5
Rough Fish-----	224	-----	78.5
Freshwater drum--	1	12.2	.8
Golden redhorse--	193	5.0 - 13.7	66.2
Hog sucker-----	23	5.0 - 13.1	6.4
Yellow bullhead--	2	8.8 - 9.8	1.1
Flathead catfish-	5	7.0 - 15.3	4.0
Mixed Minnows-----	200	1.5 - 3.0	11.6
Totals-----	513	-----	126.9

cards received from 10,000 fishermen during 1955.

The damage to the rough-fish population was determined by placing a commercial value on the estimated poundage of fish destroyed. The sum of 20.6 cents per pound was calculated by averaging the commercial sales price of rough fish taken by trot lines. The average prices charged by the commercial fishermen using nets, seines, and snag lines were excluded because these methods are not employed in Goose Creek.

The estimated creel value of fish destroyed was \$2,212 for game fish, and \$9,268 for pan fish. The estimated commercial value of rough fish destroyed was \$1,845 (table 3).

The total economic loss that resulted from the pollution of Goose Creek was estimated to be \$13,325. This is a minimum estimate of damage in that linear measurement only was used in computing the polluted segment of the stream, and no corrections were made to compensate for the increasing fish population that is

TABLE 2.--Fish population of Goose Creek in a polluted area below Island Creek on April 23, 1957

Species	Number	Number (inches)	Weight (pounds)
Game Fish-----	4	-----	5.3
Largemouth bass--	1	13.9	1.6
Smallmouth bass--	3	7.5 - 15.9	3.7
Pan Fish-----	18	-----	2.2
Rock bass-----	3	6.9 - 8.1	1.3
Longear sunfish--	15	3.0 - 5.6	.9
Rough Fish-----	44	-----	10.6
Golden redhorse--	25	5.0 - 9.0	2.1
Hog sucker-----	10	4.0 - 7.1	.8
Quillback-----	2	8.0 - 9.3	.7
Longnose gar-----	4	18.7 - 21.1	2.5
Flathead catfish--	3	12.9 - 17.8	4.5
Mixed Minnows-----	23	1.5 - 3.0	.4
Totals-----	89	-----	18.5

normally encountered when approaching the mouth of a creek. Also, muskellunge were not obtained in the shallower areas sampled, but the fish kill showed that they had been present in the deeper downstream holes.

TABLE 3.--Estimated economic loss in 16.3 miles of Goose Creek, as determined by creel value and commercial value of species killed

	Calculated number of game fish	Calculated number of pan fish	Calculated poundage of rough fish
Polluted area-----	298	1,343	780
Unpolluted area-----	2,111	8,940	9,747
Number of catchable fish destroyed-----	1,813	7,597	-----
Creel value of fish killed-----	\$2,212	\$9,268	-----
Poundage of commercial fish destroyed-----	-----	-----	8,957
Commercial value of fish killed-----	-----	-----	\$1,845
Total damage-----	-----	\$13,325	-----



The toxicity of Gymnodinium brevis does not depend on the presence of living organisms.

A RECORD OF SOME APPARENTLY NATURAL AND DRASTIC CHANGES IN THE FISH POPULATION COMPLEX OF A LARGE MASSACHUSETTS POND

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PETERS POND, 127 acres in Sandwich (Barnstable County), Massachusetts, is of glacial origin and has maximum and average depths of 54 and 25 feet, respectively. The pond is landlocked and has neither inlet nor outlet. Stratification occurs in the summer. Sand, gravel, and rubble predominate as bottom types. The pH ranges vertically from 7.4 to 6.0.

Fishing pressure has always been light, perhaps never exceeding 50 hours per acre annually, and largely centered on trout. Trout have been maintained through stocking. Some 88,345 catchable-size brook, brown, and rainbow trout were stocked between the years 1915 and 1955. Chinook and landlocked salmon (139,955; 1917-38), smelt (2,000,500; 1919-32), white perch (7,550; 1926-33), and yellow perch (1,750; 1933) were stocked as indicated by official stocking records. However, smallmouth bass must have been stocked unofficially prior to 1911.

In 1911 a survey indicated that smallmouth bass were present in small numbers and furnishing little in the way of sport. The original habitat of the smallmouth bass did not embrace New England. This same survey also reported the presence of yellow and white perch but the complete absence of the brown bullhead.

Numerous angler accounts of stomach contents of salmon caught in the 1920's and 1930's indicate the presence of an established and reproducing smelt population during this period. That such a smelt

population existed and constituted an integral segment of the fish life in the pond is attested by the fact that smelt were abundant enough to be salvaged here for stocking elsewhere during the latter half of the 1930's.

In September 1939, three fyke traps were set in this pond for a 48-hour period to check on the growth and survival of planted salmon and trout. Although no salmon or trout were taken, the following fish were: 471 smallmouth bass (5 to 16 inches), 13 large yellow perch, 2 brown bullhead (13 inches), 320 pounds of white sucker, and 25 white perch (average, 10 inches). Another fish sample, secured in 15 hours of gill netting and seining in August 1948, consisted of 20 white perch (8 to 13 inches), 20 white sucker, 3 brown trout, and 2 yellow perch. Although no direct evidence of smelt was observed during this latter survey, it was reported that reliable observers had noted their presence earlier in the year.

In September 1955, the pond was completely reclaimed for trout management and a maximum effort was expended to recover the entire fish population. Slightly more than 6 tons of fish was recovered: 85 trout (113 pounds), 1 yellow perch (0.8 pound), 7 brown bullhead (7 pounds), 60,169 white perch (2,263 pounds), 10,284 white sucker (3,397 pounds), 31,850 pumpkinseed (335 pounds), 13 eels (80 pounds), 1 smelt, and a scattering of golden shiners and Johnny darters.

The fish population complex recovered reflected cataclysmic changes involving both the introduced and the native species that apparently were self-sustaining for a number of years. Recovery of but one yellow perch (which, along with the brown bullhead, is generally conceded to be ubiquitous in Massachusetts) represented virtual extinction of this species. Seven large brown bullhead in the type of population recovered must have reflected no more than threshold numbers for perpetuation of the species. All that remained of the once-flourishing smelt population was one lone survivor. Likewise, the introduced smallmouth bass in a 16-year period had tumbled from abundance to oblivion. Evident change in the white perch popula-

tion was manifested in a decline in average size. Whereas the white perch taken in the fish samples of 1939 and 1948 consisted of specimens in excess of 8 inches in length, no more than 50 individuals of this size or better were found in the some 60 thousand recovered at reclamation. The average size of better than 95 percent of these white perch was 4.5 inches. Though it might be supposed that these fish were relatively old and stunted, straight aging revealed that they were 1-year fish spawned the preceding season.

Causation of these changes must remain a mystery. They cannot be knowingly attributed to defined factors, such as fish kills, because none are known to have occurred.



OBSERVATIONS MADE FROM AN UNDERWATER PLASTIC CAGE

In the course of investigations of the tuna and tuna baitfish in Hawaiian waters, the staff of the Bureau of Commercial Fisheries has developed a technique for making observations under water. This technique involves use of a plastic cage that can be lowered over the side of a research vessel. From the platform of the cage, a biologist wearing a skin diving suit and receiving air pumped from the deck of the vessel observes the progress of fishing operations. While the plastic cage remains under water, the vessel can proceed at speeds up to 5 or 6 knots.

Observation of fishery operations from the underwater vantage point has already produced better understanding of the behavior of some bait fish. For example, in the past many fishermen have believed that the anchovy, when thrown into the water to attract a school of tuna, would return and collect near the stern of a fishing vessel. It has been discovered, however,

that the little anchovy used in Hawaiian waters will swim rapidly away from the ship when thrown into the water. If tuna are about, the anchovies then turn and flee toward the ship, bringing with them the school of tuna. Similarly, observations have disclosed that tilapia remain close to the fishing vessel when they are thrown into the water and thus, because they do not go out and attract the school of tuna, are not so good a bait as the little anchovy.

From the underwater cage it has been possible to watch tuna rising from deep water to get the anchovy bait. The visibility under water around Hawaii extends to a depth of at least 150 feet. The horizontal distance for such observations may be even greater.

This new technique is expected to produce valuable information as to the best bait species, how and when to throw bait, and where to start "chumming."

"Sunburn" among fish may be the result of a dietary deficiency.

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